The Synthesis of Conformationally Restricted Amino Acids and Peptide Mimics Using Ring-Closing Metathesis

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A thesis

submitted in partial fulfilment

of the requirements for the degree

of

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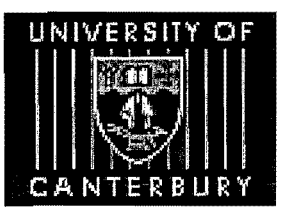
by

James Gardiner



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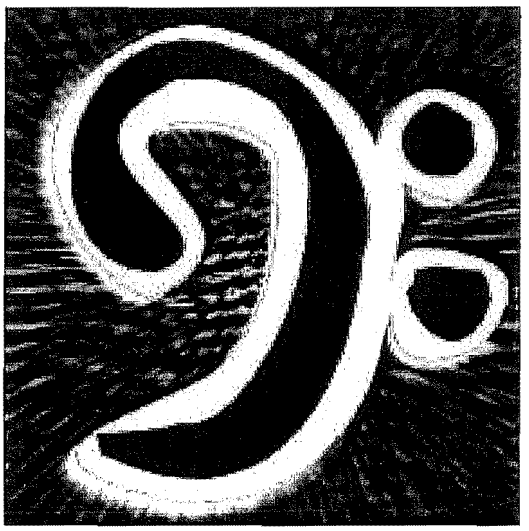

Metathesis Album cover

"What's important to me: Ideas, not trends."

- Bernhard Fischer, Metathesis

"That which we persist in doing becomes easier to do, not that the nature of the thing itself is changed, but that our power to do is increased."

- Ralph Waldo Emerson

"Don't brag about your lightning pace, for slow and steady won the race."

- The Tortoise and the Hare

"You don't have to be a fantastic hero to do certain things to compete. You can be just an ordinary chap, sufficiently motivated to reach challenging goals."

- Edmund Hillary

"No success can compensate for failure in the home."

- David O. McKay

WORK IN THIS THESIS HAS APPEARED IN THE FOLLOWING PUBLICATIONS

"Synthesis and X-Ray Structure of a 1,2,3,6-Tetrahydropyridine-Based Phenylalanine Mimetic.", Abell, A. D., Gardiner, J., Phillips A. J., Robinson W. T. *Tetrahedron. Lett.* **1998**, *39*, 9563-9566.

"Synthesis of substituted cyclohexenyl-based β-Amino Acids by Ring-Closing Metathesis.", Gardiner, J., Abell, A. D. *Org. Lett.* **2002**, *4*, 3663-3666.

"A Diastereoselective Synthesis of the Tetrahydropyridazinone Core of 2-Oxo-1,6-diazobicyclo[4.3.0]nonane-9-carboxylate-based Peptidomimetics Starting from (*S*)-Phenylalanine.", Gardiner, J., Abell, A. D. *Tetrahedron Lett.* **2003**, *44*, 4227-4230.

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ABSTRACT

Peptidomimetics have found wide application as biostable, bioavailable, and often potent mimics of natural peptides. Examples of peptidomimetics have been isolated as natural products, synthesized as libraries from novel subunits, and designed on the basis of X-ray crystallographic studies and through an intricate knowledge of the biological mode of action of natural peptides. They offer challenging synthetic targets and are increasingly important medicinal agents and biological probes.

Chapter One introduces the fields of peptidomimetics and peptidomimetic design, with relevant examples taken from biochemistry, synthesis, and medicinal chemistry used to illustrate important concepts. The sources of peptidomimetics, along with common tools used for the conformational restriction of peptides, is then reviewed. Ring-closing metathesis (RCM), an important new method for the formation of carbocyclic rings is then reviewed, and discussed in terms of its application towards the synthesis of conformationally restricted peptidomimetics. This section emphasises the importance of RCM as a new and versatile tool for use in peptidomimetic synthesis.

Chapter Two describes the enantioselective synthesis of the conformationally constrained, α-substituted tetrahydropyridine, and piperidine peptide mimics, **2.9** and **2.12** respectively, via RCM. The solid-state conformations of **2.9** and **2.12** are examined and their application as potential *cis*-amide bond mimics discussed. Further derivatisation of **2.9** gave dihydroxy analogues **3.13**, and dibromo analogues **2.14**. A novel bicyclic lactone **2.16** was also formed upon crystallisation of the major isomer of **2.14**. The enantiomeric purity of a key intermediate **2.7** was analysed, and determined to be >95%, confirming the stereoselectivity of a key alkylation step.

Chapter Three describes the enantioselective synthesis of the conformationally constrained, α-substituted dehydroproline, and proline peptide mimics, **3.17** and **3.28**, by RCM, in a manner similar to that detailed in Chapter Two. Use of the ruthenium methyl-butylidene catalyst **1.41** was required for RCM to proceed. Comparison of the solid-state conformation of **3.17** to that of **2.9**, reveals the two molecules adopt a positive torsion angle about the amide bond in the solid state, despite possessing opposite stereochemistry.

Chapter Four describes the first enantioselective synthesis of the phenylalanine-based tetrahydropyridazinone **4.13**, and its conversion to the 2-oxo-1, 6-diazobicyclo[4,3,0]nonane-9-carboxylate dipeptide-mimetic scaffold **4.14**, an important component of extended β-strand mimetics. Conformationally constrained bicyclic templates of this type have been designed as key components in inhibitors of serine proteases such as thrombin.

Chapter Five describes a versatile ring-closing metathesis (RCM) approach to the synthesis of the unsubstituted *trans*-cyclohexenyl β-amino acids **5.27** and **5.28**, compounds that serve as key intermediates in the synthesis of β-peptides that adopt stable helical conformations in solution. Optically active (-)-**5.27** and (-)-**5.28** were prepared using Evans chiral auxiliary chemistry. The solid-state structure of (+)-**5.40a** was used to determine the absolute stereochemistry of a key intermediate (+)-**5.25**, used in the preparation of (-)-**5.27** and (-)-**5.28**. *Cis*- and *trans*-cyclohexenyl β-amino acids **5.34** and **5.36** respectively, compounds containing an α-substituent, are synthesised by an allylation/alkylation sequence, the order of which defines the absolute stereochemistry of the product. This second class of cyclic β-amino acids represents a new and important addition to the family of compounds. Optically active allylglycine hydrochloride (-)-**5.53** was synthesised via the stereospecific alkylation of the Ni(II)-BPB-glycine complex **5.50**, with allyl bromide.

Chapter Six describes a convenient and versatile ring-closing metathesis approach to the synthesis of *trans*-aminocyclopentenylcarboxylic acids (+)-**6.19** and (+)-**6.20**, from *L*-methionine. Compounds of this type are key intermediates in the synthesis of β-peptides that adopt stable helical conformations in solution. The *trans*-cyclopentenyl β-amino acid **6.27** was prepared by an allylation/alkylation sequence similar to that described in Chapter Five, the order of which defines the absolute stereochemistry of the product.

Chapter Seven describes a ring-closing metathesis approach towards the synthesis of six- and seven-membered cyclic lactams derived from functionalised α- or β-amino acids. RCM of the allylglycine-derived diene **7.24** at 85° C resulted in exclusive formation of the 7-membered lactam **7.25**, while RCM of **7.24** at 100° C, gave a 1:1 mixture by ^{1}H NMR of **7.25** and the 6-membered lactam analogue **7.26**. Preparation of a,a-disubstituted amino acid-derived lactams required a substituent on the allyl amide nitrogen for RCM to proceed. Dienes of type **7.37** and **7.43**, that are either unsubstituted or substituted at the α-position, are prepared by an alkylation/allylation sequence similar to that described in Chapter Five. RCM of **7.37** and **7.43** gave the 7-membered lactam **7.38** and the 6-membered lactam **7.45** respectively. Alkylation on the lactam nitrogen of **7.38** gave dipeptide **7.39**, illustrating the ability of these compounds to be incorporated into peptide sequences

ABBREVIATIONS

$[\alpha]_D$	specific rotation
δ	chemical shift
Boc	*tert*-butoxycarbonyl
brs	broad singlet (in NMR)
Cbz	benzyloxycarbonyl
COSY	correlation spectroscopy
CIGAR	constant time inverse-detected gradient accordian rescaled long-range HMBC (in NMR)
d	doublet (in NMR)
dd	doublet of doublets (in NMR)
DIEA	*N*,*N*-diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide
EA	ethyl acetate
EDCI	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
ee	enantiomeric excess
EI	electron impact ionisation (in mass spectrometry)
equiv.	equivalents
ES	electrospray ionisation (in mass spectrometry)
FTIR	Fourier transform infrared
h	hour(s)
HIV	human immunodeficiency virus
HMBC	heteronuclear multiple bond correlation (in NMR)
HOBT	1-hydroxybenzotriazole
HRMS	high resolution mass spectrometry

HSQC	heteronuclear single quantum correlation (in NMR)
Hz	hertz (in NMR)
J	coupling constant
LiHMDS	lithium hexamethyl disilazide
LRMS	low resolution mass spectrometry
m	multiplet (in NMR)
Micro.	Microanalysis
min	minute(s)
mp	melting point
MTPA-Cl	α-methoxy-α-(trifluoromethyl)phenylacetic acid chloride
NMO	4-methylmorpholine-*N*-oxide
NMR	nuclear magnetic resonance
PE	petroleum ether (bp 50-70 °C)
ppm	parts per million
RCM	ring-closing metathesis
rt	room temperature
s	singlet (in NMR)
t	triplet (in NMR)
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
Z	benzyloxycarbonyl

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Thanks to all the academic and technical staff I have come to know over my time in the department. In particular, John Blunt (NMR), Bruce Clark (mass spec), Rob McGregor (glass blowing), and Professor Ward Robinson, Dr Jan Wikaira, Dr Jon Cannon and Glen Fern (X-ray crystallography) for contributing to the research in this thesis.

Thanks to the Abell Group, past and present, for your friendship and endless hours of entertainment. From the well-versed 'originals' of 637, to the estrogen-packed youngsters of 839, I say a heart-felt 'thanks' for all the good times. Its scarey to think that I might be a product of my environment. To all the other students I have met in the department over the years, I say thanks, and look forward to hearing of your progress.

To my friends outside of University. Yes I have finally finished. No more 'nearly'. I can now remove the ( ) from around the Dr and assume the title of 'Dr J'.

Thanks to my parents, Ron and Jenny, for their love and support throughout my student experience, and also to the rest of my family for their encouragement.

Finally, I would like to thank my lovely wife Misiona, for her love and patience during my pursuit of 'science stuff', especially during this last year. Its all about chemistry baby!!

CHAPTER ONE

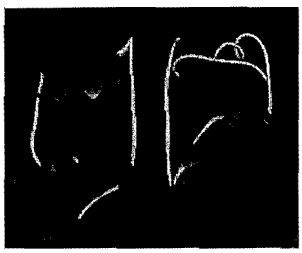
INTRODUCTION

1.1. Peptides

Peptides are highly flexible molecules that are capable of adopting multiple conformations, many of which are biologically unimportant. The conformation of a peptide is, therefore, critical to its biological activity and function. A simple example is that of angiotensin, a biologically active octapeptide that plays an important role in the regulation of blood pressure. Angiotensin affects blood pressure directly by inducing constriction of blood vessels, and indirectly by the affecting the release of aldosterone to induce sodium ion and water retention. Instances of cardiovascular diseases such as hypertension and heart failure have been attributed to an imbalance in this system. Cleavage of the *N*-terminal aspartate residue of angiotensin leads to a decrease in activity indicating that this residue is important to the overall binding properties of the parent peptide.

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe

Angiotensin



Insulin

A more complex example is that of insulin, a molecule consisting of two polypeptide chains linked by disulphide bonds, and the primary peptide involved in the regulation of glucose metabolism. Released in the pancreas, insulin acts on nearby liver cell receptors to regulate the breakdown of glycogen to glucose. A deficiency of insulin can lead to an increase in blood glucose levels and the onset of diabetes mellitus, a disease rated as one of the most widespread and serious health concerns in the world today. While these two peptides differ in structure, they each possess the ability to modulate a biological process by binding to their associated receptors in a unique way. The binding, and hence function, of these molecules is therefore directly related to their unique bioactive conformation. The diverse nature of the amino acid residues that make up peptides allows for an almost unlimited exploration of conformational space within any given molecule. As such, a wide range of structurally diverse biologically active peptides and proteins have been identified and characterised in recent decades. These peptides have been found to act as pivotal

components in the regulation of a myriad of physiological processes. Through interacting with a specific receptor, these peptides control a series of vital functions such as metabolism, cell-cell communication, digestion, immune response, respiration, pain, reproduction, and behaviour. Disease states associated with these processes are often linked to a disruption in the function of the biologically active peptides and the receptors or enzymes on which they act. It is therefore of no surprise that biologically active peptides are of enormous interest to medicine in the treatment of disease.

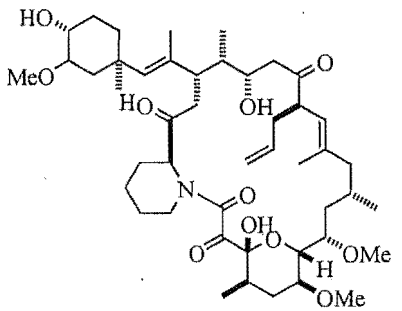
1.2. Peptidomimetics

Peptides and their analogues have long been used in medicinal chemistry as therapeutic agents against a range of pathological conditions generally characterised by a disruption of the interactions between an enzyme substrate or messenger and their targets, enzymes or receptors. However the presence of hydrolysable amide bonds means that peptide drugs possess the inherent disadvantages of a short half-life, poor oral availability, and poor pharmacokinetics. As such, considerable effort has been expended on finding modified or non-peptide surrogates for biologically active peptides. Surrogates of this typ ere more commonly designated a peptidomimetic.

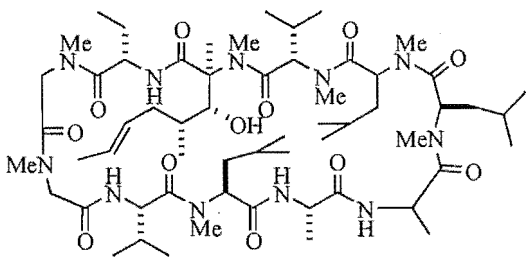
"A peptidomimetic is defined as a substance having a secondary structure, as well as other structural features, analogous to that of the parent peptide that allows it to displace the original peptide from its receptor or enzyme. As a result, the effects of the original peptide are either inhibited (antagonist), or duplicated (agonist)."[1]

A successful peptidomimetic, while possessing all these characteristics, must also be sufficiently non-peptidic to overcome the problems of degradation and bioavailability associated with peptides, whilst exhibiting minimal side effects. This enhanced therapeutic profile has made peptidomimetics attractive targets in the search for more effective treatments of disease. As such, peptidomimetics offer challenging synthetic targets and have embraced much of what is modern medicinal and organic chemistry.

There are two common sources of peptidomimetics, natural sources, and rational drug design. Natural products have long served as a significant source of important peptidomimetics, with mass screening of animal, plant, microbial, and fungal extracts providing an invaluable guide to the identification and isolation of biologically active compounds. In some cases the parent peptide itself can be used to treat disease e.g. insulin in the treatment of diabetes, while in others, natural products derived from alternate sources serve to promote or inhibit key biological functions. Examples of natural product peptidomimetics include cyclosporin A (CsA), FK-506, and ω-conotoxin MVIIA (Figure 1.1). Cyclosporin A and FK-506 are generally viewed as immunosupressants, with the macrocyclic peptide CsA having emerged as the principal immunosuppressive agent for solid organ transplants.[2] The marine natural product ω-conotoxin MviiA is currently in clinical trials as a powerful agent against intractable pain, and with its non-addictive characteristics is touted as a future alternative to morphine.[3]



tacrolimus (FK-506)



cyclosporin A

ω-conotoxin MviiA

Figure 1.1.

In recent decades, rational design has come to the fore in the development of peptidomimetics as biological probes and medicinal agents. Advances in the area of combinatorial chemistry has allowed for the generation of vast libraries of organic compounds, which range from purely peptidic to non-peptidic in nature, from which mass screening can, and has, identified new lead compounds. Solid phase chemistry and mass screening technology have been critical to the preparation and identification of these new

lead compounds. The activity of these lead compounds can be further optimised through the identification and variation of key structural and functional elements of the original peptidomimetic platform. In the same way, natural products can also be screened and their activity refined through structure-activity relationship studies. Technological advances in modern nuclear magnetic resonance (NMR) spectroscopy, computer modelling and x-ray structure analysis of peptides has allowed researchers to understand and visualise the topochemical, conformational, and electronic properties of a peptide ligand and its corresponding receptor. As such, a *rational-based* approach can be taken to the identification of potential pharmacophores, compounds that map all the structural and electronic elements of the bioactive ligand, which are then used as a basis for peptidomimetic design. Many peptidomimetic compounds have been developed as successful therapeutic agents by means of this process.

1.3. Peptidomimetic Design

The design and synthesis of peptidomimetics is a complex process. What follows is a overview of techniques used in peptidomimetics design.

1.3.1. Conformational Restriction

An extended or randomly orientated peptide or protein is generally devoid of biological activity. The biological function of a given peptide or protein is achieved through the spatial arrangement of the peptide backbone, which is in turn defined by the primary amino acid sequence. This highlights a key concept in peptidomimetics research, that is *conformation defines biological activity*. Enhanced activity can be achieved through the mimicking of the bioactive conformation of a native substrate, with the incorporation of additional structural elements designed to stabilise and make rigid this desired bioactive conformation. These rigid structural features are designed to ensure the correct positioning of key functional groups in order to optimise hydrogen bonding, electrostatic, and hydrophobic interactions between the peptidomimetic ligand and the receptor. As such,

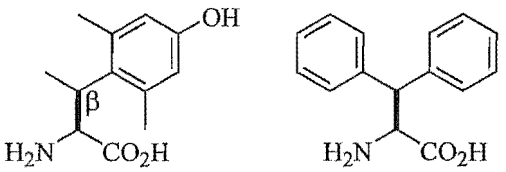
peptidomimetics can essentially be preorganised into a bioactive conformation by the incorporation of rigid structural elements. These are designed to lower the entropy cost exhibited by a peptidomimetic upon binding to the receptor, thereby increasing its affinity and enhancing its overall biological activity What follows is a summary of the strategies used to introduce conformational restriction into peptidomimetic design.

1.3.2. Introduction of Substituents

The synthesis of a peptidomimetic is not always an easy process as the requirements mentioned above mean that the chiral pool of natural amino acids can no longer serve as the sole source of starting materials. Consequently, new chiral building blocks are required for use in peptidomimetic design and synthesis.

Side chain modification

An established method for the preparation of conformationally restricted chiral building blocks involves the modification of naturally occurring amino acid side chains. The incorporation of unnatural or modified amino acids, or the replacement of a residue with its optical isomer, provides useful information regarding possible turn sites, while modification of a side chain gives clues as to its role in the bioactive conformation. The introduction of sterically demanding substituents limits the free rotation of the amino acid residue (Figure 1.2). An example is the modification of tyrosine by the introduction of methyl groups at the 2', 6' and β-positions (**1.1**).[4] This has been shown to favour bioactive conformations and has been used to study the effects of restricted rotation of aromatic side chains in the interior of proteins (e.g. bovine pancreatic trypsin inhibitor),[5] and peptide-protein complexes (e.g. oxytocin and neurophysin),[6] as well as other bioactive peptides such as methionine-enkaphilin.[4] Compound **1.2**, a constrained analogue of phenylalanine has been incorporated into potent peptidomimetic ligands of the angiotensin II receptor.[7]

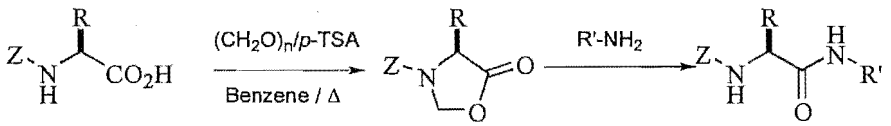


1.1 **1.2**

Figure 1.2.

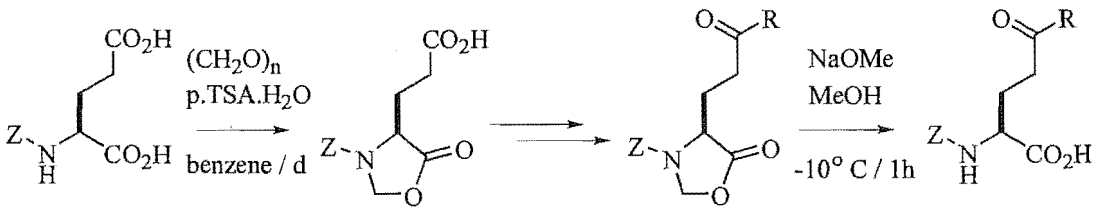
α,α-Disubstituted Amino Acids and the Self-Regeneration of Stereocentres.

Another powerful method in the synthesis of modified peptides has been the use of 5-oxazolidinones to prepare a range of novel chiral building blocks for use in this regard. Although reported as early as 1904, it was not until the 1950's that this class of compound began to be explored in the area of synthesis. In particular, work done by Ben-Ishai demonstrated that 5-oxazolidinones could be prepared from the reaction of acyl amino acids with paraformaldehyde (Scheme 1.1).[8]



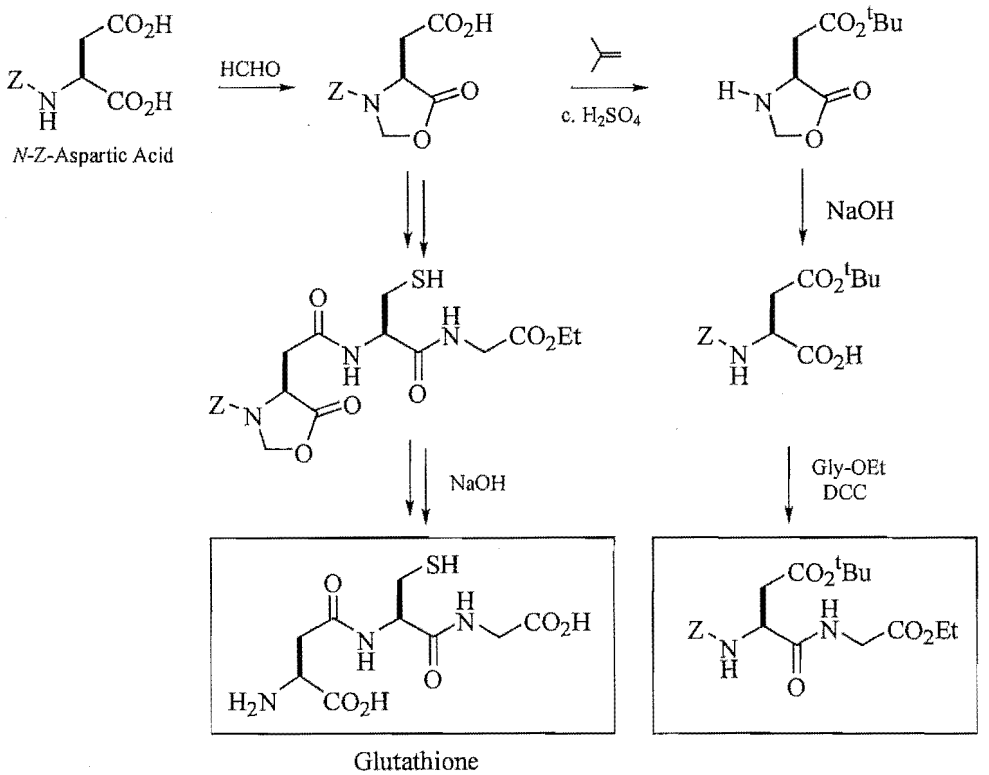
Scheme 1.1

In this case the reactivity of an oxazolidinone towards an amine was used as a method for amide bond formation. Ben Ishai's procedure for the synthesis of 5-oxazolidinones was later used by several groups to affect selectivity of peptide couplings and modify amino acid side chains. 5-Oxazolidinones were used in peptide synthesis to selectively protect either the α-carbonyl, or the side-chain carboxylic acid groups, of aspartic and glutamic acids. This allowed for modification of either of the carboxyl groups to be carried out selectively. Bartlett's differential protection strategy for the manipulations of aspartic and glutamic acids (Scheme 1.2),[9] and Itoh's synthesis of glutathione (Scheme 1.3),[10] illustrate this principle well. Bartlett used an oxazolidinone to selectively protect the α-carbonyl groups of aspartic and glutamic acids to allow manipulation of the side chain carboxylic acid.



Scheme 1.2.

Itoh's synthesis demonstrated that 5-oxazolidinones could be used to selectively react an amino acid with either the α-carbonyl or side-chain carboxyl group of aspartic acid. This methodology was used to prepare pure samples of glutathione, an important thiol-containing tripeptide found in almost all aerobic biological species.



Scheme 1.3.

The use of oxazolidinones has thus become an important tool in the modification of amino acid side chains, with the functionality itself playing no role in subsequent reactions. In more recent years 5-oxazolidinones have found a more general role in the area of peptidomimetics. In the 1980's Karady *et al.*,[11] and Seebach *et al.*,[12,13] first described the

enantioselective synthesis of α,α-disubstituted amino acids through the stereospecific alkylation of amino acids. Conformational studies revealed that the presence of a methyl substituent at the α-position of a given amino acid residue imposed a significant restriction on the available conformational space adopted by that residue. Increased resistance to proteolytic cleavage has also made α,α-disubstituted amino acids useful in this regard.

Seebach *et al.* subsequently pioneered a general methodology for producing enolates of α-amino or α-hydroxy acids such that subsequent alkylation yielded non-racemic products. This simple, yet broadly applicable synthetic principle is known as the "Self Regeneration of Stereocentres" (SRS) and is outlined in Figure 1.3.[14] The general principle utilises a chiral starting material possessing two functional groups, where only one stereogenic centre is allowed to react with an aldehyde to form an acetal, with preference for one isomer. Annihilation of the original stereogenic centre and concomitant formation of a trigonal centre yields an intermediate, which due to the stereogenic centre of the acetal, is chiral. Subsequent reaction at the trigonal centre proceeds diastereoselectively under the influence of this temporary stereogenic centre, with cleavage of the acetal unit leading to a product where one of the substituents of the starting material has been replaced by a new one. The reaction proceeds by dissociative, enantioselective substitution at a centre of chirality and without the need for a chiral auxiliary – hence the name "self-regeneration', with hydrolysis of the acetal leading to the regeneration of the aldehyde.

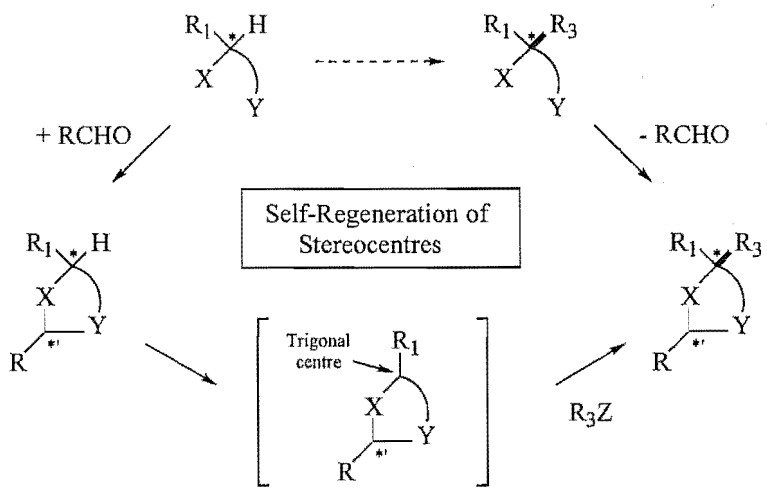


Figure 1.3. The principle of the Self Regeneration of a Stereogenic centre.

Application of the principle of SRS to the chiral pool of proteinogenic amino acids subsequently allows for the preparation of a wide range of α,α-disubstituted amino acids for use as building blocks in peptidomimetics design (Figure 1.4).

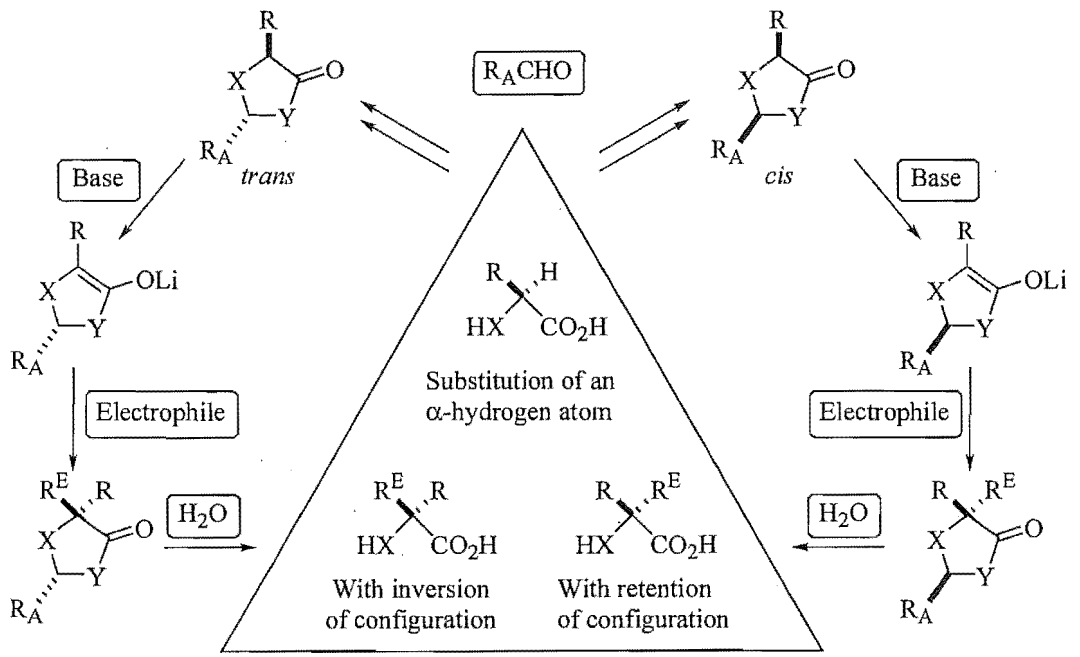
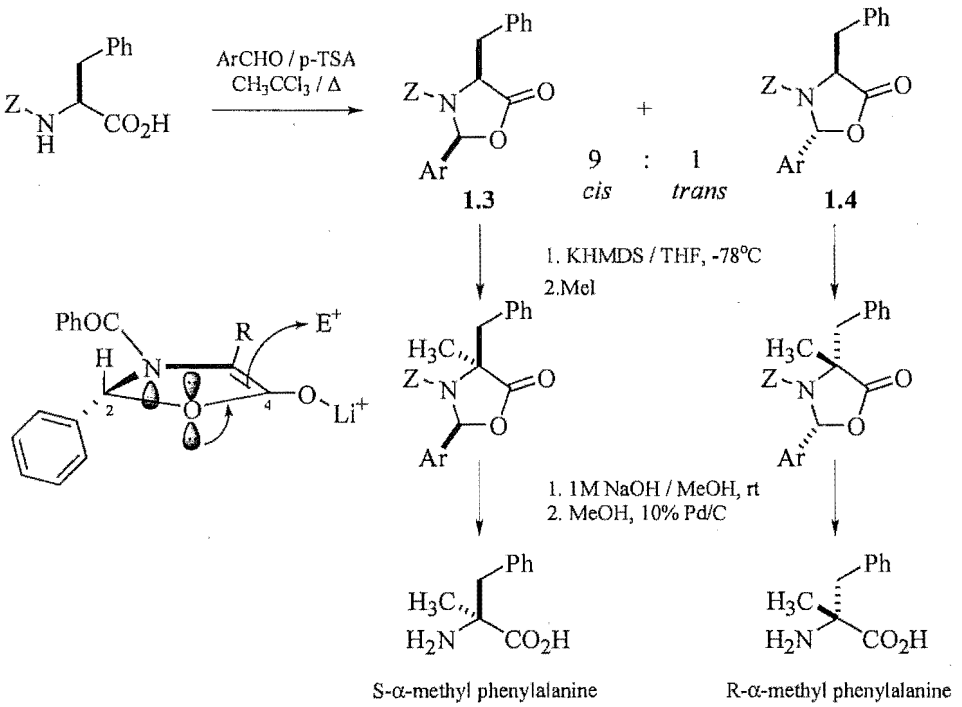


Figure 1.4.

Protection of an amino acid in the form of an oxazolidinone (Y=O), or imidazolidine (Y=NMe), results from a thermodynamically controlled reaction of the carboxylic acid derivative with an aldehyde. The occurrence of either the *cis* or *trans* form of the resulting heterocycle strongly depends on the nature of the *N*-acyl group and the aldehyde. For example, *N*-benzoyl oxazolidinones derived from benzaldehyde give predominantly *trans* heterocycles, while those derived from pivaldehyde give predominantly *cis* heterocycles. Hence, acetals derived from (*S*)-amino acids can be produced with either an (*R*) or (*S*) configuration at the acetal centre. Subsequent deprotonation results in the formation of a heterochiral enolate that can undergo diastereoselective alkylation, at the α-carbon, to form enantiomerically pure products. Hydrolysis of the acetal results in a wide variety of chiral α,α-disubstituted amino acid building blocks for use in the synthesis of peptidomimetics.

The principle of SRS and its extensive applications in synthesis has since been expertly reviewed.[14]

As an example, *N*-Z-phenylalanine is condensed with an aryl aldehyde to give a 9:1 mixture of 2-aryl-4-benzyl-oxazolidinones **1.3** and **1.4**, where the *cis* isomer **1.3** is the major product (Scheme 1.5). Separation of the isomers and subsequent deprotonation of each in the presence of MeI, followed by hydrolysis, gave enantiomerically pure samples of *R* and *S* α-methyl phenylalanine. Significantly, 5-oxazolidinones of this type, substituted at the 2 position, direct alkylation of the subsequent enolate at the face opposite this substituent.



Scheme 1.4. The enantioselective synthesis of α-methyl phenylalanine.[11]

5-Oxazolidinones have been used in this manner during the synthesis of selective neurokinin receptor ligands of type **1.5**, and bombesin receptor ligands of type **1.6**, where the α-methyl substituent imparts conformational stability *in vivo* (Figure 1.5).[15] Additionally, α,α-disubstituted amino acids themselves have been found to be biologically

active, with one example being (*S*)-α-methyldopa **1.7**, a compound that exhibits hypotensive activity and inhibits the decarboxylation of (*S*)-dopa by mammalian decarboxylase.[12]

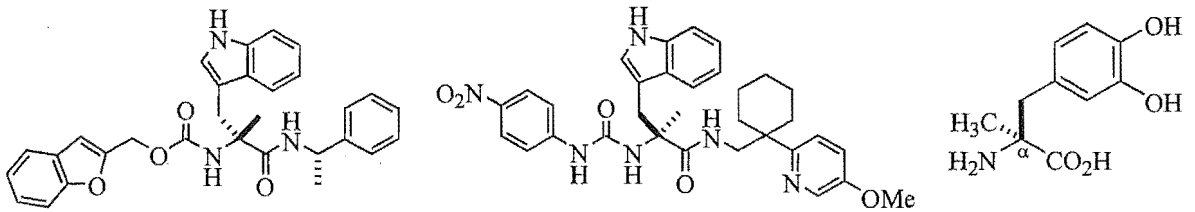
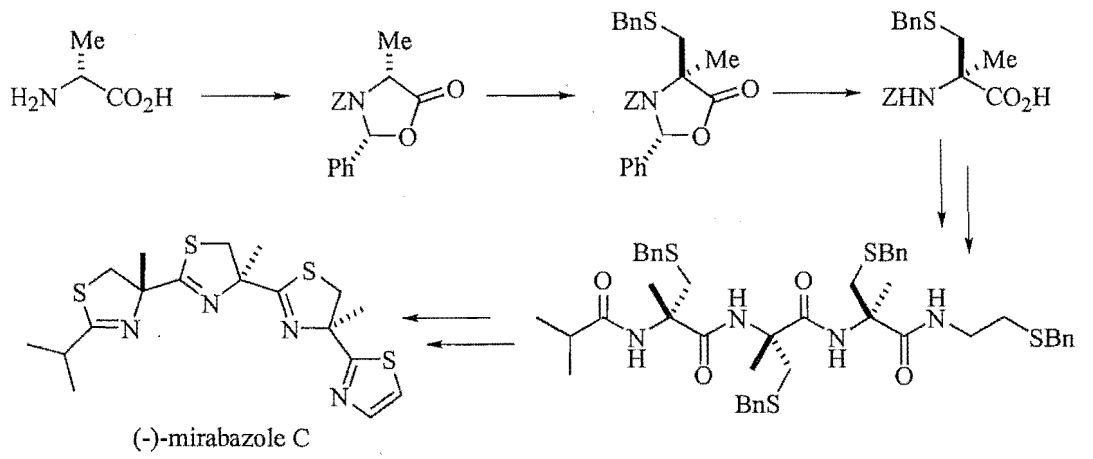


Figure 1.5.

Conversely, natural product peptidomimetics can also be synthesised using this methodology. An example is (-)-mirabazole C, a tetrathiazone/thiazol alkaloid isolated from the blue/green alga *Scytonema mirabile*, that has shown selective cytotoxicity against solid tumours and inhibitory activity against HIV-1 protease. Akaji *et al.* utilised chiral oxazolidinone chemistry to prepare chiral methylcysteine subunits for use in the synthesis of the target natural product (Scheme 1.5).[16]



Scheme 1.5. Reagents and Conditions: i. $PhCH(OMe)_2$, $BF_3.Et_2O$, -15° C, ii. $LiN(Et)_2$, $BnSCH_2Br$, iii.LiOH.

α,α-Disubstituted amino acids have subsequently emerged as very important chiral building blocks in peptidomimetic synthesis, and are used to induce a defined conformational restriction in a range of peptide and peptide-like compounds..

1.3.3. Peptide Backbone Modification

Modification of a peptide backbone generally involves the exchange of units within a peptide chain with electronically and/or sterically equivalent units, as well as the incorporation of additional units. These modifications lower the peptide character of the mimic, thereby increasing proteolytic stability, while retaining key steric or electronic properties of the parent peptide. Some common examples of peptide backbone modification are outlined in Figure 1.6.

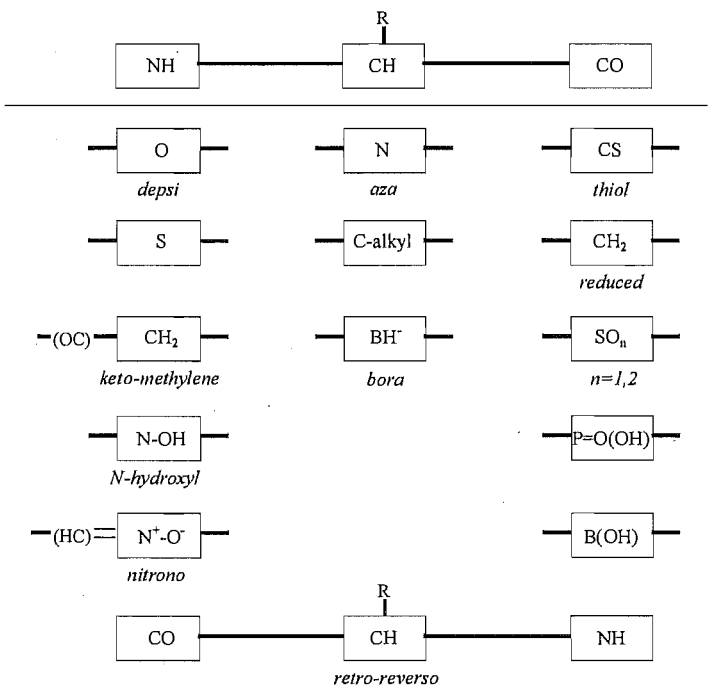


Figure 1.6.

A key aim here is to modify the parent peptide so that it adopts a preferred bioactive conformation. Often this involves constraining a peptide bond to adopt either a *cis*- or *trans*-amide geometry. Pauling and Corey's pioneering work on the structure of peptides

and proteins revealed that the peptide bond is planar and rigid, such that the hydrogen of the substituted nitrogen is nearly always *trans* to the carbonyl oxygen, due to the partial double character of the amide bond. Delocalisation of the nitrogen electrons limits free rotation about the amide bond thereby limiting the rate of interconversion between the *cis*- and *trans*-coplanar forms (Figure 1.7).

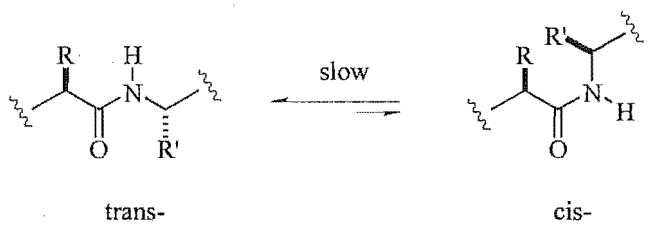


Figure 1.7.

The conformation of an amide bond is therefore crucial to the binding of an extended peptide-based ligand to a receptor. In a typical amide bond the *trans*-conformation is favoured over the *cis*-conformation by approximately 10 kcal/mol, due to less steric interaction between adjacent amino acid side chains, with extended peptide ligands often seen binding to a receptor with a preference for one or other of the conformations. In many cases, biologically active peptides bind with a *cis* conformation despite this being energetically disfavoured.

An example is the Phe-Pro cleavage site in substrates of HIV protease, a site unique to retroviral proteases. Initial studies of HIV protease suggested that a *cis*-conformation was preferred at this position in substrates that bind in the active site of the enzyme. This led to the development of the potent HIV protease inhibitor JG-365, a compound in which the peptide backbone has been modified to include a hydroxyethylamine isostere in place of the key phenylalanine residue (Figure 1.8). X-ray crystal studies of JG-365 bound to HIV protease revealed that the inhibitor binds to the active site in a pseudo-*cis* conformation about the C-C bond adjacent to the proline residue. The torsion angle about this bond is 11°, indicating the conformation to be essentially planar. However, while JG-365 is a potent inhibitor *in vitro* it fails to inhibit HIV protease in cellular assays, due to its largely peptidic character. Studies done in this laboratory have shown that further modification of

the peptide backbone of JG-365, to incorporate a tetrazole moiety as a *cis*-amide bond isostere, constrains the peptide mimic to adopt a similar conformation to that of JG-365. The tetrazole moiety constrains the peptide such that the torsion angle about the C-tetrazole bond is approximately 13°, a value in close agreement with that found in the equivalent C-C bond in the bioactive conformation of JG-365. Consequently, compounds of type **1.8**, that contain a modified tetrazole backbone, have been shown to be good inhibitors of HIV protease.[17,18]

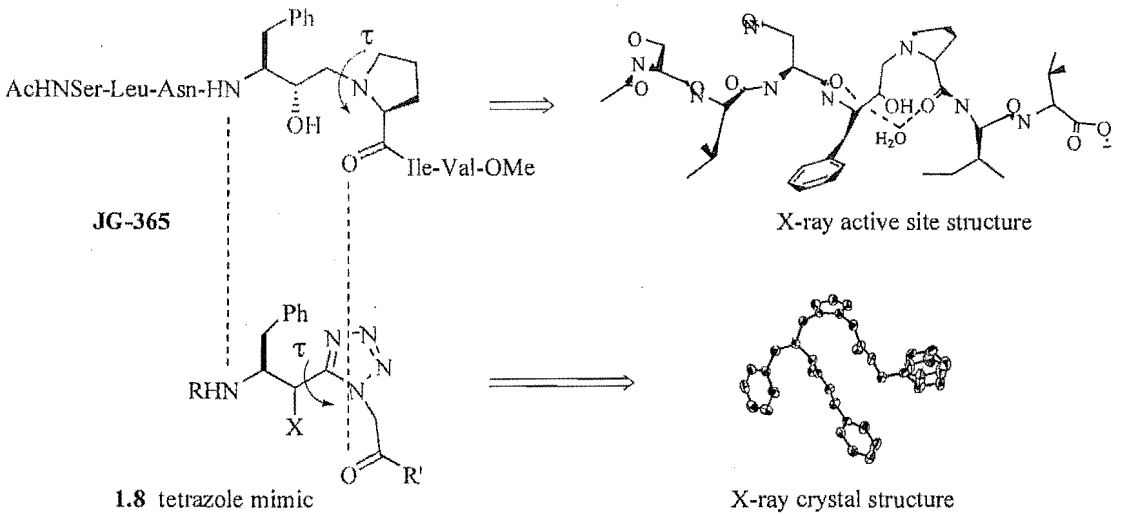


Figure 1.8.

Another prominent example can be found in the use of 1,2-disubstituted and 2,5-disubstituted pyrroles of type **1.9** and **1.10**, as *cis* and *trans* amide mimics respectively. (Figure 1.9)

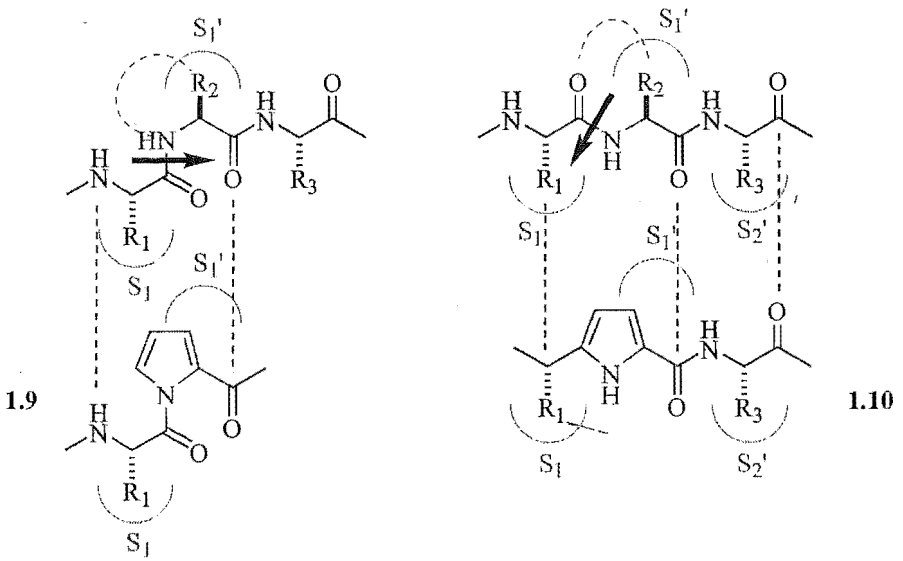


Figure 1.9.

Latent reactivity can be incorporated into compounds of this type in the form of a hydroxymethyl moiety that can undergo reaction to form a highly electrophilic azafulvene intermediate. Peptidomimetics of this type were developed in this laboratory as mechanism-based inhibitors of α-chymotrypsin, with reaction of the azafulvene intermediate with an enzyme active site nucleophile resulting in a covalently bound enzyme-inhibitor adduct (Figure 1.10).

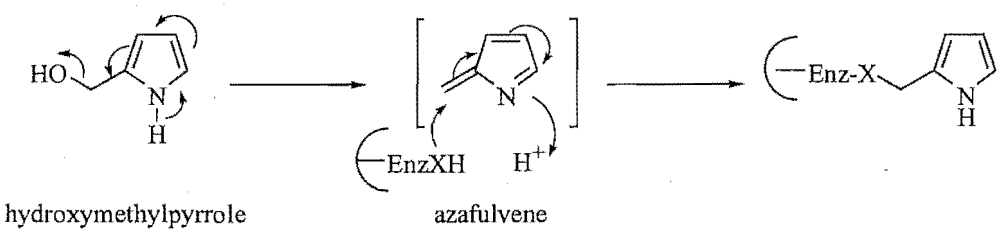
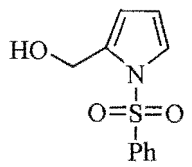
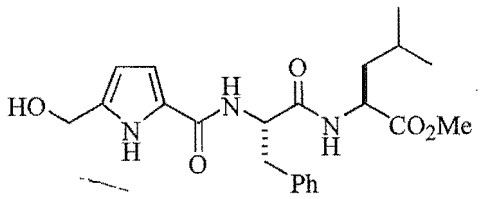


Figure 1.10.

Peptidomimetics of type **1.11,** and **1.12,** incorporate electron-withdrawing group at the C-2 and N-positions respectively, and are designed to deactivate the pyrrole ring system and suppress the formation of an azafulvene intermediate. Upon cleavage during enzyme hydrolysis these compounds release these electron-withdrawing groups are cleaved leading to azafulvene formation and the release of latent reactivity.



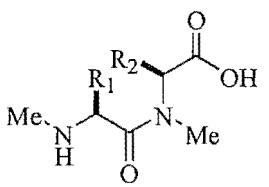
1.11



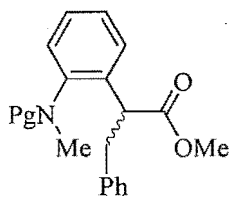
1.12

Other examples of *cis* and *trans* modified peptide isosteres are shown in Figure 1.11. *N*-Alkylated amides such as **1.13** are well known for adopting a *cis*-amide conformation with *N*-methylated amino acids found in many naturally occurring bioactive peptidomimetics. The ortho-substituted benzene mimic **1.14** has been incorporated into potent inhibitors of HIV protease,[19] as has another popular *cis*-amide mimic, the *cis*-epoxide **1.15**.[20] The *trans*-alkene isostere **1.16** has been utilised in a number of peptidomimetics, with derivative **1.17** having been incorporated into a potent neurotensen binder,[21] while the fluorinated analogue **1.18** has been identified as an inhibitor of dipeptidyl protease IV.[22]

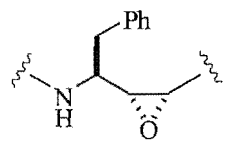
cis-amide bond mimics



1.13

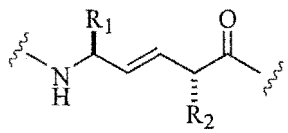


1.14

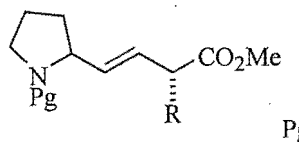


1.15

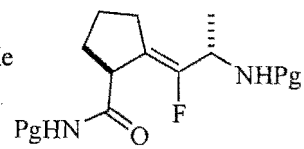
trans-amide bond mimics



1.16



1.17

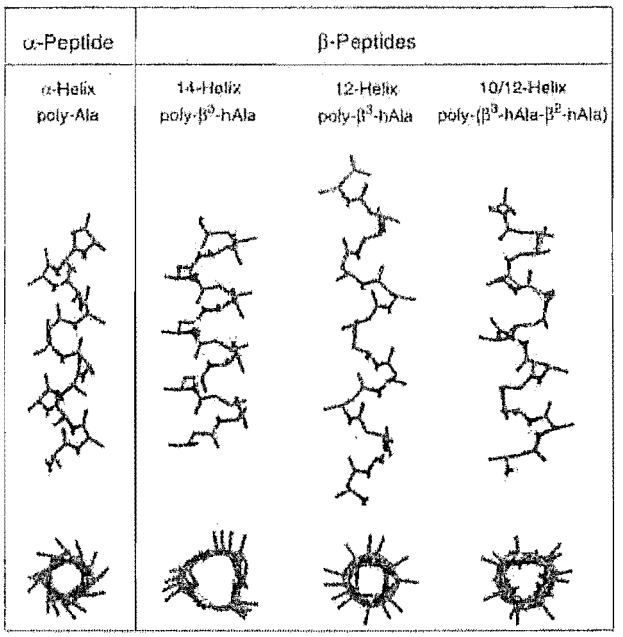


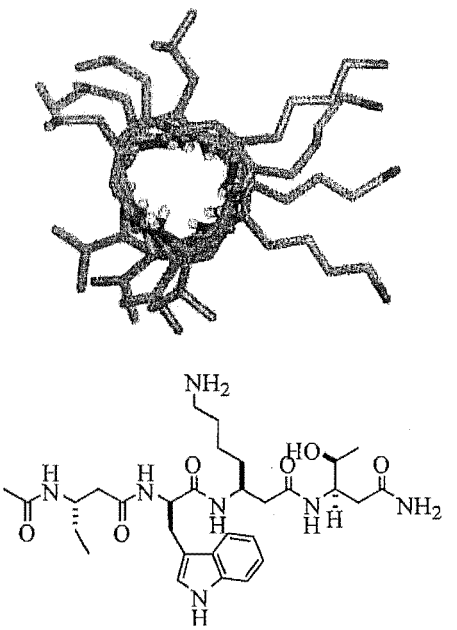
1.18

Figure 1.11.

In recent times, a significant method of backbone modification has been the incorporation of β-amino acids into peptide chains. Oligomers composed of β-amino acids have since been shown to adopt stable and distinct helical structures in solution, a phenomenon previously thought to be unique to α-amino acids (Figure 1.12). An additional property of β-peptides is their enhanced biological stability towards proteolytic enzymes, making them ideal for use as peptidomimetics. The development of β-peptides as peptidomimetic

therapeutics has since become the focus of intense research. Recent examples include a range of α-helical β-peptides that possess potent cell-lytic activity, and the tetra β-peptide **1.19**, a powerful binding ligand of somatostatin (see Chapters Five and Six for a more detailed discussion).





1.19

Figure 1.12.[23]

1.3.4. Ring -Formation

The most common, and perhaps the most important, tool in peptidomimetics design is the use of cyclization to restrict conformational mobility. The introduction of rigid bridges of varying lengths between different parts of a peptide, can improve potency by locking the ligand into a preferred bio-active conformation thereby increasing its affinity for its associated receptor or enzyme active site. The exclusion of conformations that are capable of inhibiting other enzymes also enhances the specificity of the ligand for its target. Commonly, two adjacent amino acid residues are involved in bridging, however there are many other sites where this is possible (Figure 1.13).

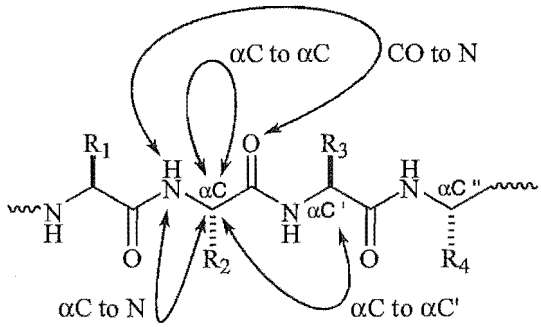
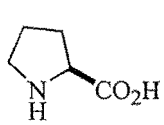
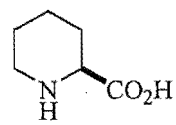


Figure 1.13. Bridging sites for the introduction of conformational stabilising rings.

Two naturally occurring examples of conformationally restricted cyclic amino acids are proline and pipecolic acid. These residues contain a carbon bridge linking the amine nitrogen to the α-carbon, thereby restricting rotation about the N-αC bond. The result is a unique kink or bend in a peptide wherever these residues are incorporated with many important structural proteins and bioactive peptides containing key proline or pipecolic acid subunits (see Chapters Two and Three for a more detailed discussion).



L-proline

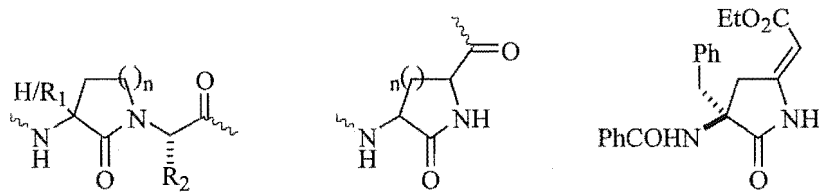


L-pipecolic acid

Cyclic Peptidomimetics

Well-known examples of peptidomimetics that incorporate bridging rings are Freidinger lactams, compounds that contain a bridge between the amide nitrogen of one residue and the α-carbon of an adjacent residue. Lactams of this type are commonly used to restrict the rotation about a key amide bond in order to stabilise a preferred bioactive conformation. Compounds of type **1.20** have been used to stabilise a *trans* amide bond geometry in the development of inhibitors of the renin-angiotensin system, a key component in the regulation of blood pressure (Figure 1.14).[24] α-Substituted derivatives of this type have found use as versatile dipeptide isosteres.[25] with similar compounds, incorporating varying ring sizes, having also been developed as inhibitors of Hepatitis C

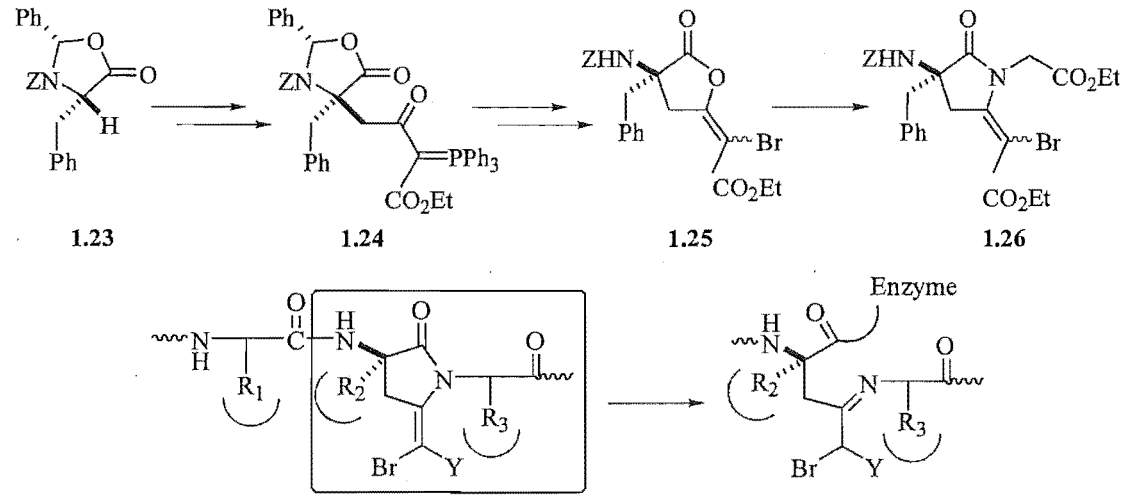
Virus NS3 protease (see Chapter 7 for a more detailed discussion). Conversely, compounds of type **1.21** represent examples where bridging between adjacent amino acid residues leads to the stabilisation of a *cis*-amide bond geometry.[26] Toniolo *et al.* recently described the application of these compounds towards the construction of new helices, large-ring cycle correlates and nanotubes.[27] Compounds of type **1.22**, developed by the author in this laboratory, have similarly been designed as *cis*-amide bond mimics in this regard.[28]



1.20 **1.21** **1.22**

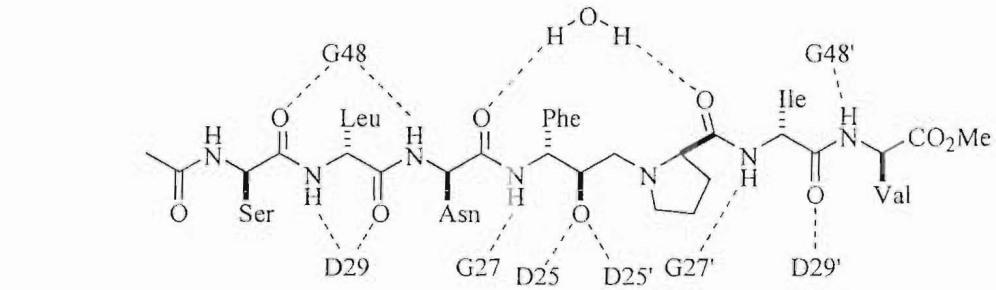
Figure 1.14.. Conformational stabilising rings used in peptidomimetics design.

The introduction of cyclization to restrict conformational mobility also amends itself to the incorporation of latent reactivity. An example is the cyclic enamino ester dipeptide analogue **1.26** (Scheme 1.6), developed in this laboratory as a new class of inhibitor of serine proteases.[29] The synthesis of these compounds involves the bromine mediated lactonisation of a key keto-acid phosphorane **1.24**, derived from a chiral oxazolidinone **1.23** (refer to Section 1.3.2 Introduction of Substituents), to form the bromo enol lactone **1.25**. Compounds of this type are known to be mechanism-based inhibitors of serine proteases, with subsequent treatment of **1.25** with an amino acid affording the dipeptide analogue **1.26**. Enzyme mediated cleavage of the lactam ring of **1.26** results in the release of latent reactivity and the covalent binding of the inhibitor to the enzyme active, thereby inhibiting its mode of action.



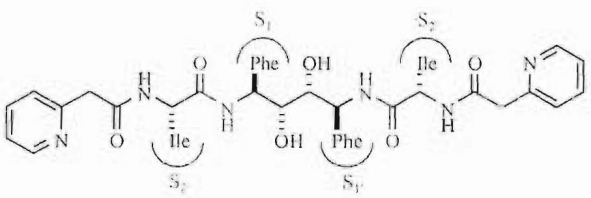
Scheme 1.6.

Another impressive example of the use of rings to constrain conformation can be seen in the computer aided *de novo* design of cyclic urea inhibitors of HIV protease.[30] The first high resolution X-ray crystal structures of this aspartyl protease, bound to the hydroxyethylamine inhibitor JG-365, confirmed that the enzyme was C_2 symmetric in nature and contained a tetra-coordinated structural water molecule that linked the bound inhibitor to the flexible 'flaps' of the HIV protease dimer (Figure 1.15).[31] The presence of this water molecule is a unique feature of the retrovirus protease. As such, inhibitors of type **1.27** (Figure 15b), containing a C_2-axis of symmetry and using a diol as the transition-state mimetic, were developed and found to be potent inhibitors of HIV protease. However, diols of this type proved to be highly insoluble and possessed very poor bioavailabilty. Consequently, Lam *et al.* utilised 3-D computer techniques to develop a novel scaffold possessing the key diol moiety and the necessary structural motifs to bind the active site residues and the structural water molecule. The result was the introduction of cyclic ureas of type **1.28** (Figure 15c), as new and potent and selective inhibitors of HIV protease that exhibit high bioavailability and good human pharmacokinetics.[30] Figure 15c shows the enhanced interactions brought about by the cyclic nature of the mimic in constraining key functional groups to adopt a key bioactive conformation.

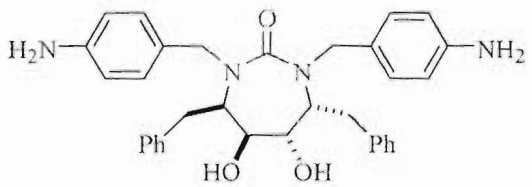


JG-365

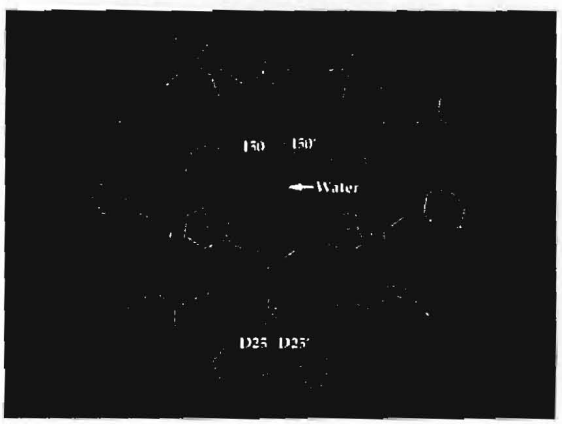
a)

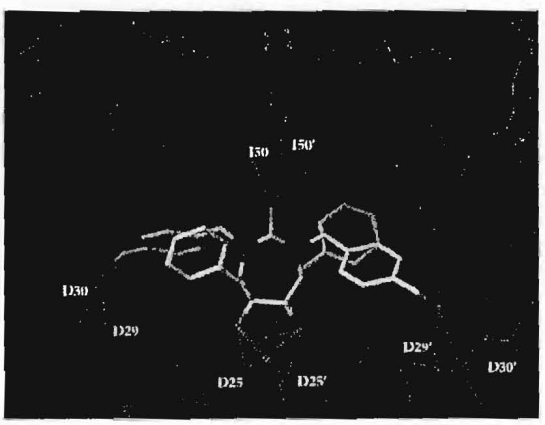


1.27



1.28





b) c)

Figure 1.15.[30] a) Hydrogen-bonding observed in the crystal structure of JG-365 complexed to HIV protease. b) Distance geometry model of **1.27** docked to HIV protease showing the extended binding conformation and hydrogen bonding interactions. c) X-ray crystal structure of **1.28**-HIV protease showing enhanced hydrogen bonding interaction.

Other examples of peptidomimetics incorporating rigid ring structure designed to stabilise bioactive conformations are shown in Figure 1.16. These include mimics that bridge within a single amino acid residue (**1.29**[32] and **1.30**[33]), between two adjacent side chains (**1.31**[34] and **1.32**[35]) or adjacent backbones (**1.33**[36]), between a backbone and a side-chain (**1.34**[37]), or incorporate both the backbone and the side-chain units through the introduction of multicyclic systems, as in the case of the potent ACE inhibitor **1.35**.[38]

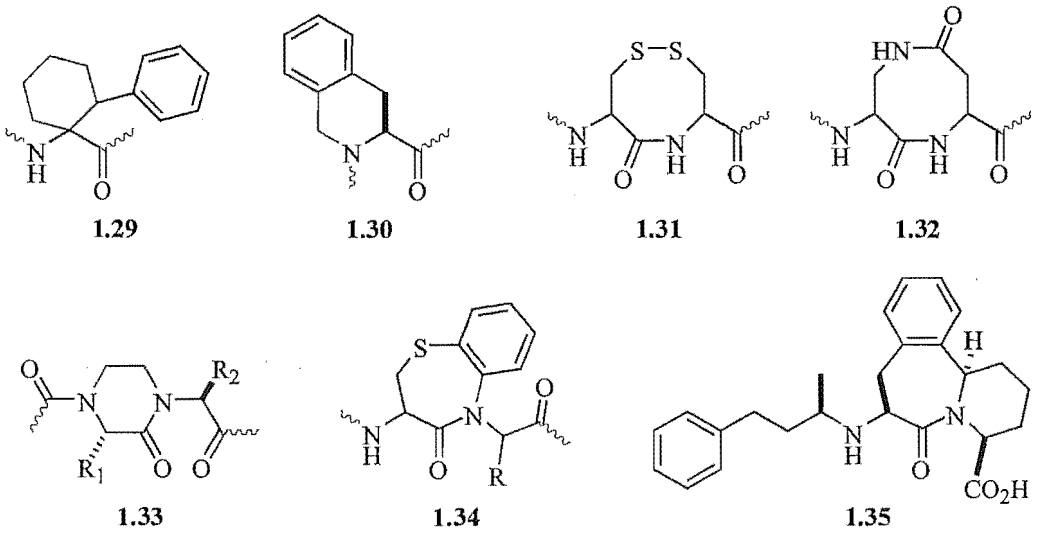


Figure 1.16.

Macrocyclic Peptidomimetics

Many biologically active macrocyclic peptides are found in nature. Their biological activity, in contrast to their acyclic counterparts, is due in part to the inherent conformational restriction provided by the cyclic system. The restricted conformation of macrocyclic peptides offers enhanced binding selectivity with receptors as the bioactive conformation is obtained from a smaller population of conformers. This is true of all constrained peptidomimetics and offers a distinct entropic advantage over analogous acyclic peptides. Compounds **1.36** and **1.37** are examples of synthetic macrocyclic peptidomimetics where the non-adjacent residues are linked to bring them close in space and induce biological activity (Figure 1.17). Both **1.36** and **1.37** are potent inhibitors of HIV protease and display enhanced metabolic stability relative to acyclic inhibitors.[39] Rational design of simplified analogues of somatostatin, a macrocyclic peptide hormone that regulates the release of growth hormone, has led to compounds showing similar potency to the parent peptide, including **1.38** where a key recognition loop is constrained to induce receptor recognition.[40]

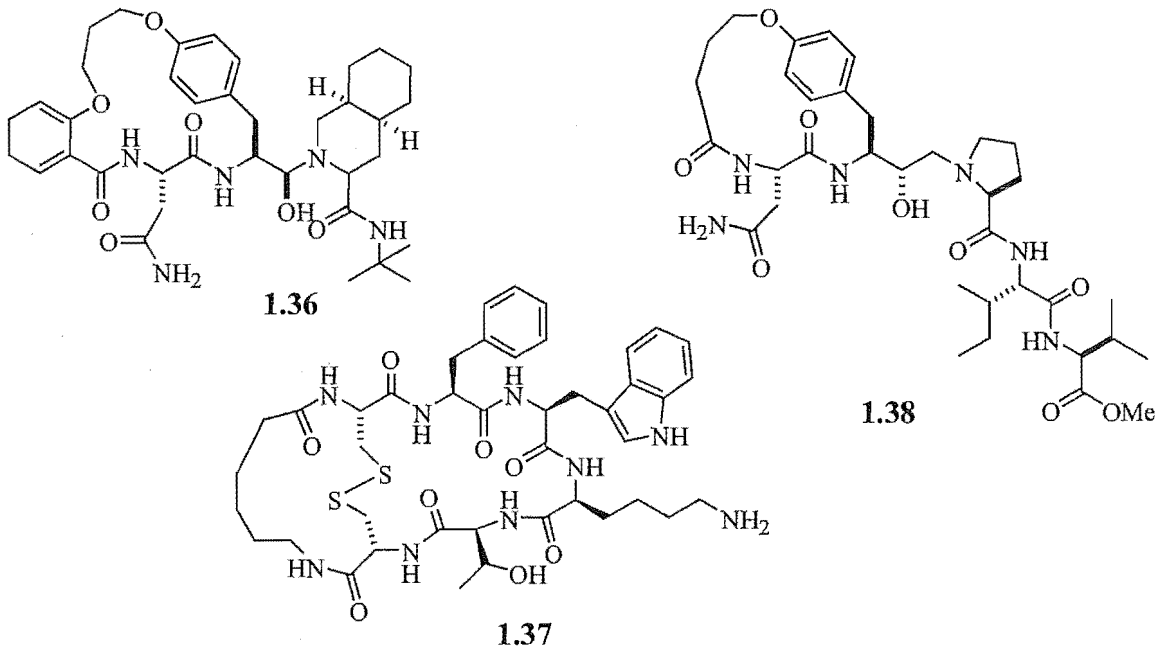


Figure 1.17.

What follows is a discussion of a general method for the introduction of rings into both peptide and non-peptide compounds.

1.4. Ring–Closing Metathesis as a Method of Ring Formation

In recent years olefin metathesis has emerged as a powerful new tool in organic synthesis and has seen widespread use in the preparation of modified peptides and peptidomimetics. The power of this reaction lies in its ability to transform a carbon-carbon double bond, a functional group that is relatively unreactive towards many reagents. Olefin metathesis involves the bringing together of two carbon-carbon double bonds (or olefins), in the presence of a transition metal catalyst, to form a new carbon-carbon double bond. This can be carried out at or near room temperature, in a variety of media (organic or aqueous), from starting materials that bear a variety of functional groups. Ring-closing metathesis (RCM) is an example of this reaction in which the initial olefins are contained within the same acyclic starting molecule (Figure 1.18.). As such, RCM has emerged as a powerful

new method for the synthesis of carbocyclic rings and has since been applied extensively towards the synthesis of a wide range of conformationally constrained and cyclic peptidomimetics.

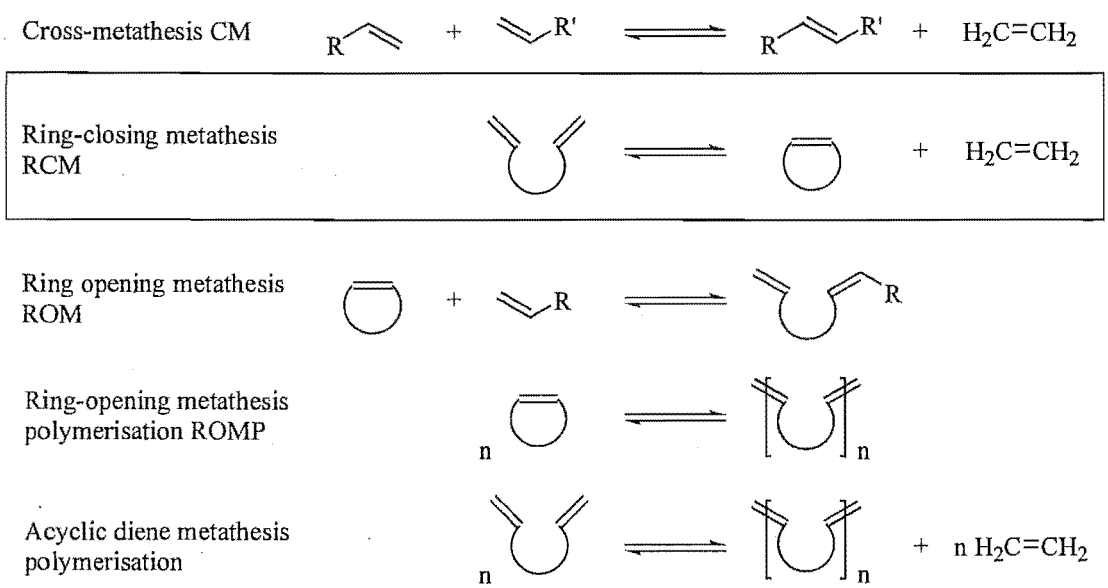
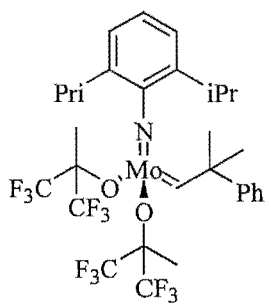
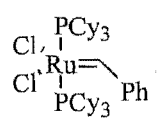


Figure 1.18.

Many of the advances in olefin metathesis can be directly attributed to the improvements made in metal-carbene catalysts and the development of well-defined catalyst systems. In particular, work by Schrock, and Grubbs, has resulted in the molybdenum catalyst **1.39**,[41-43] and ruthenium catalyst **1.40**,[44-47] seeing widespread use in organic chemistry in recent years (Figure 1.19).



1.39 Schrock's catalyst



1.40 Grubbs' catalyst

Figure 1.19.

A significant benefit of these catalysts is their functional group tolerance, with cyclisation occurring in the presence of free alcohols, carbonyl groups and other potentially reactive functionalities. Functional groups of this type are often incompatible with traditional methods of ring-closure and require extensive protection. Applications of **1.39** and **1.40**, and related catalysts, in organic chemistry and peptidomimetic synthesis have been the subject of a number of reviews,[48-51] with Grubbs' catalyst **1.40** being named chemical of the year in 1998.

RCM is most easily understood when considering the cyclisation of a simple diene by means of a metathesis – an exchange of groups between the two arms of the molecule. A schematic mechanism for the RCM reaction involving **1.40** is shown in Figure 1.20.

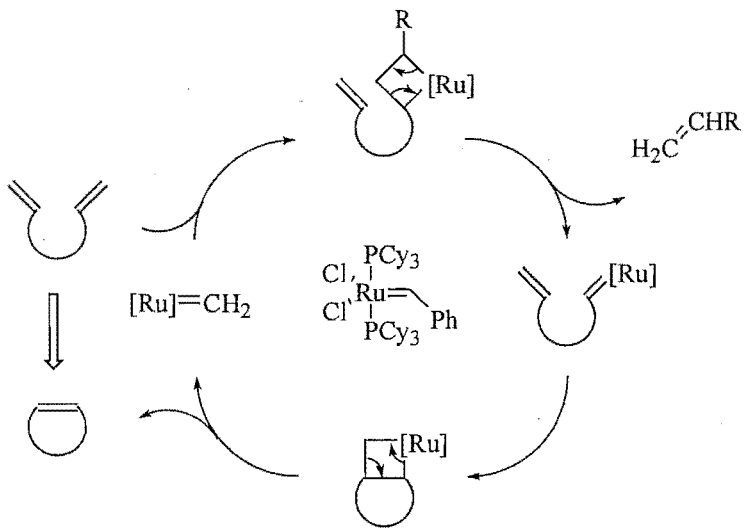


Figure 1.20.

First, carbene **1.40** adds to one of the alkenes of an acyclic diene by means of a [2+2] cycloaddition, to give a metallocyclobutane. The same reaction then happens in reverse to give either the starting materials or, by cleavage of the other two bonds, a new carbene complex with styrene (R=Ph) as a byproduct. Next, an intramolecular [2+2] cycloaddition occurs to link the two arms of the molecule and produce a second metallocyclobutane. This decomposes in the same way as the first to give a third carbene complex and the newly cyclised product. This new carbene complex then attacks another molecule of

starting material and the cycle is repeated, with the exception that ethylene is now lost instead of styrene in all remaining cycles.

Since the introduction of Schrock's catalyst **1.39**, and Grubbs' catalyst **1.40**, many other catalysts have been developed with the aim of improving both reactivity and selectivity. For example, **1.39** has the major disadvantage of being air- and moisture sensitive, while the excellent profile of **1.40** is hampered by its thermal instability and low reactivity towards substituted double bonds. As such, ruthenium based catalysts have received the greatest attention due to their high selectivity, and ability to be used under less stringent conditions. Incorporation of large electron-donating ligands and small electron withdrawing halogens was found to lead to more active catalysts. The subsequent exchange of one PCy_3 unit of **1.40** with *N*-heterocyclic carbene ligands led to "second generation" metathesis catalysts with superior reactivity and increased stability (Figure 1.21).[52-54] The highly reactive alkylidene complexes **1.42** and **1.43** are over a thousand times more reactive than **1.40** and efficiently catalyse reactions of substrates which do not react with catalyst **1.40**, including α-β-unsaturated olefins. In addition, it was noted that, in many cases, catalysts with extended carbene tethers (i.e. **1.41**) effected RCM of more hindered substrates by exhibiting increased co-ordination during formation of the intermediate metallocyclobutane.[47,48] It was also observed that addition of $Ti(i\text{-}PrO)_4$ to reactions where difficulties were encountered cyclising substrates with potential metal coordinating functionalities adjacent to the alkene, led to an increase in the rate and yield.[55]

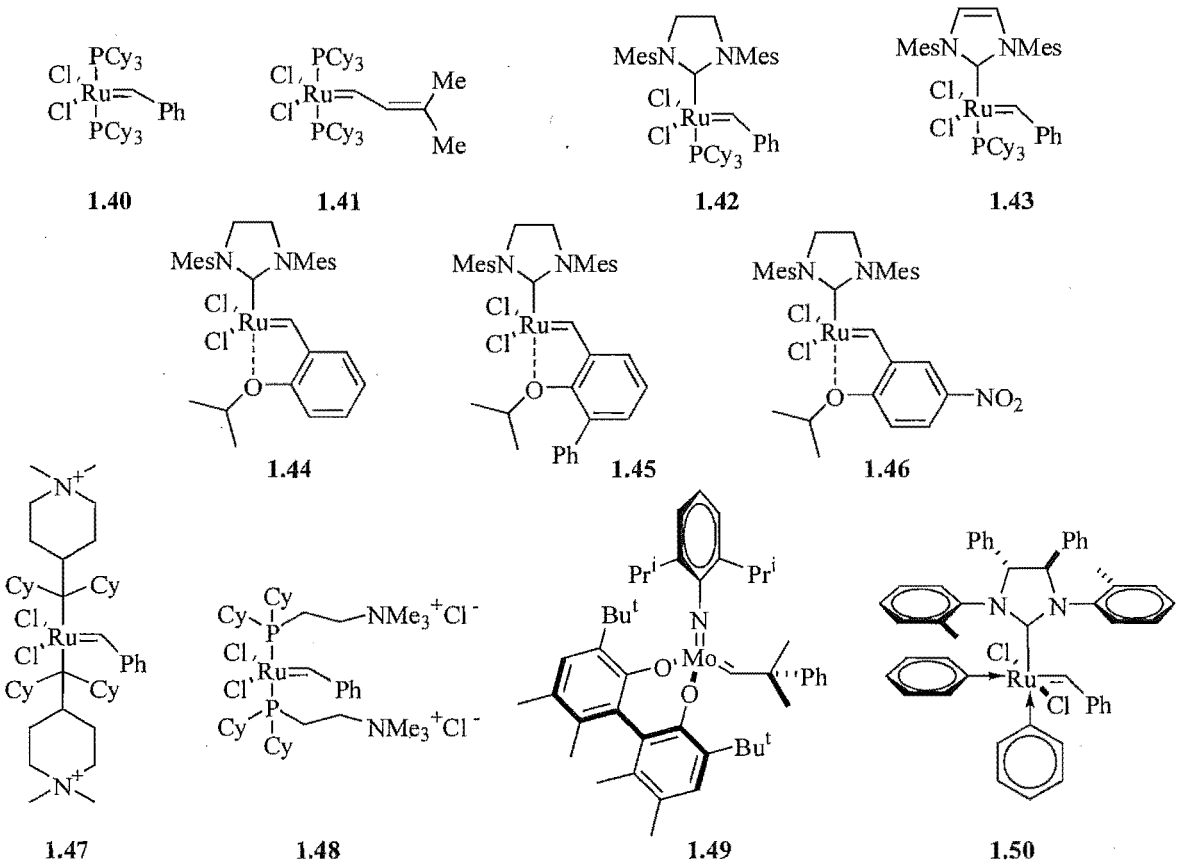


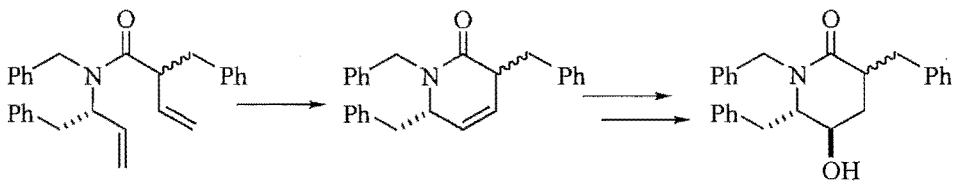
Figure 1.21.

Hoveyda recently established catalysts of type **1.44**, as remarkably robust complexes that promote olefin metathesis by a "release-return" mechanism.[56,57] This non-phosphine catalyst possesses a greater reactivity towards electron-deficient olefins than does **1.42**. The fact that the ruthenium carbene **1.44** is air stable, can be easily purified by standard silica gel chromatography and can be recycled after the reaction are particularly appealing facets of this chemistry. Blechert and Wakamatsu have also very recently shown that replacement of the isopropoxybenzylidene ligand of **1.44** with BINOL,[58] or biphenyl-based benzylidene,[59] results in a large improvement in activity. As a result complex **1.45** is not only much more reactive than **1.44**, but also the second generation Grubbs catalyst **1.42**. Grela *et al.* have shown that the Hoveyda-type catalyst can be significantly improved by changing not only the steric, but also the electronic environment of the Ru-chelating isopropoxy fragment. The introduction of the strongly electron-withdrawing NO_2 group onto the 2-isopropoxybenzylidene ring of **1.44** leads to complex **1.46** which is just as stable

as **1.44** but dramatically more reactive.[60] This observation suggests that decreasing the electron density on the oxygen atom of the isopropoxy fragment of **1.44** results in an increase in catalytic activity. Catalysts that allow RCM in methanol and water (**1.47** and **1.48**) have also recently been developed. The phosphine ligands of these catalysts contain quaternary ammonium salts that give enhanced solubility, and activity, in protic solvents. Asymmetric RCM using catalysts **1.49** and **1.50** has also been described but has yet to see widespread use.[61]

The use of ring-closing metathesis in organic chemistry has resulted in over 2500 examples being reported in the literature over the past 8 years. RCM of nitrogen-containing compounds, with applications towards the synthesis of heterocycles, alkaloids and peptidomimetics, has subsequently been the focus of much research and has been expertly reviewed.[48] What follows are some recent examples of the use of RCM in peptide and peptidomimetic synthesis, followed by some general applications of olefin metathesis in organic synthesis.

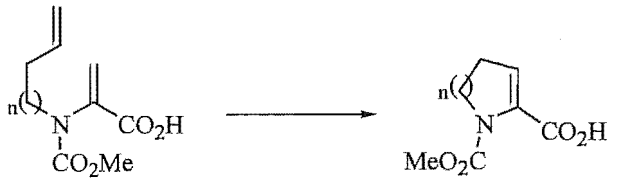
Research in this laboratory utilized **1.40** in the preparation of 5-hydroxypiperidinones of type **1.51** (Scheme 1.7). These compounds are based on the well-studied C_2-symmetirc cyclic ureas such as DMP 450 (**1.28**), and known to be potent inhibitors of HIV protease.[62]



1.51

Scheme 1.7.

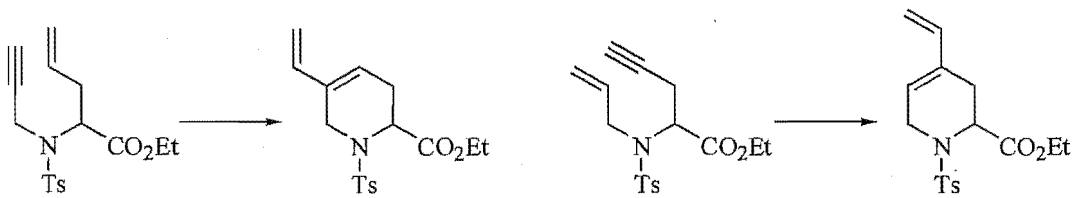
Thorstensson *et al.* used **1.42** to prepare proline isosteres of type **1.52**, for incorporation into potential inhibitors of thrombin (Scheme 1.8).[63]



1.52

Scheme 1.8.

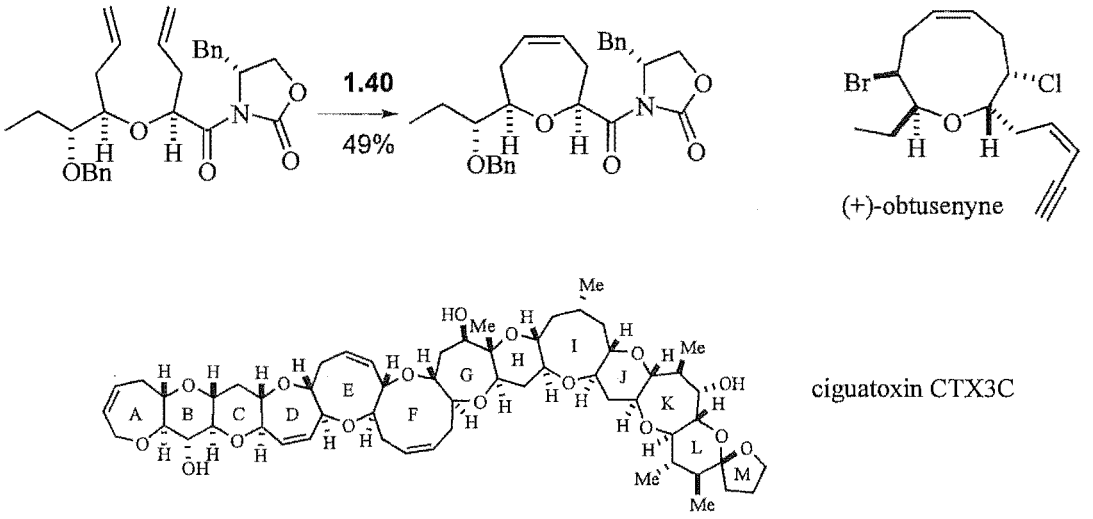
Enyne metathesis, a process by which an alkene and an alkyne are reacted to form a diene, has also seen development in recent years.[64] An example is the use of **1.40** by Kotha in the preparation of **1.53** and **1.54**, compounds utilised in the synthesis of highly constrained, and unusual, α-amino acid building blocks and peptides (Scheme 1.9).[65]



1.53 **1.54**

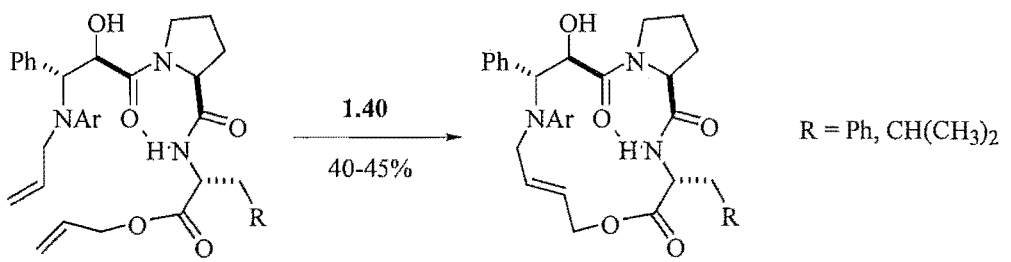
Scheme 1.9.

Crimmins and Emmitte demonstrated the use of RCM in the synthesis of α,α-*cis* and *trans* disubstituted medium ring ethers, a common structural feature of many ladder ether marine toxins such as brevetoxins and ciguatoxins (Scheme 1.10).[66] This same methodology was used in the synthesis of 9-membered ring ether (+)-obtusenyne. Hirama *et al.* later used RCM as a key step in the total synthesis of ciguatoxin itself.[67]



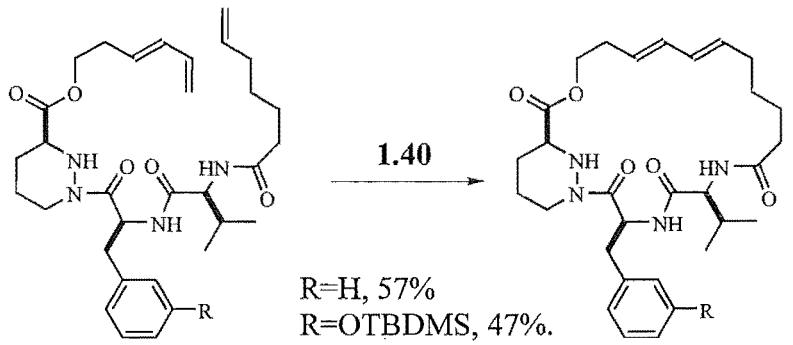
Scheme 1.10.

Iqbal *et al.* utilised RCM in the synthesis of type VI β-turn mimics, a unique member of β-turn family containing an s-*cis* peptide bond (Scheme 1.11). The understanding of the conformation of the type VI-β-turn is crucial to the development of inhibitors of HIV protease.[68]



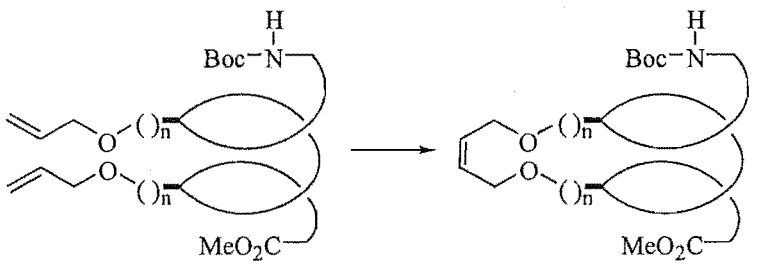
Scheme 1.11.

Wagner *et al.* used RCM for the critical macrocyclisation step in the synthesis of macrolide analogues of the potent immunosuppresent sanglifehrin (Scheme 1.12).[69] Significantly, this is one of the first examples of a macrocycle containing a conjugated diene, being prepared by RCM.



Scheme 1.12. **1.40**, R=H, 57%, R=OTBDMS, 47%.

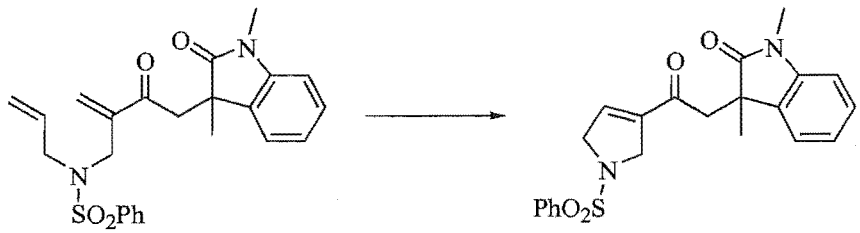
Grubbs and Blackwell illustrated the ability to covalently cross link short peptide sequences in order to initiate helix formation in short chain peptides (Scheme 1.13).[70] The extraordinary functional group tolerance of olefin metathesis is of particular use in this regard.



Scheme 1.13. 20mol% **1.40**, n=1 85%, n=2 90%

Similarly, general use of RCM throughout organic synthesis has led to a number of interesting and innovative applications being reported.

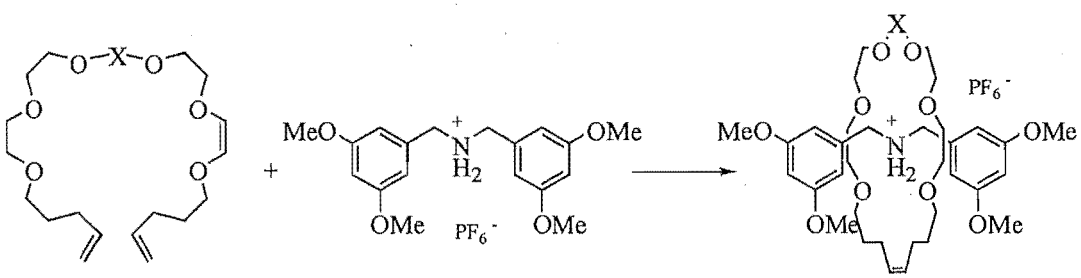
Microwave technology has been applied to a number RCM reactions leading to shorter reaction times and lower catalyst loading. Grigg *et al.* showed that RCM of **1.55**, to give **1.56**, could be achieved in one minute with 100% conversion (Scheme 1.14).



1.55 **1.56**

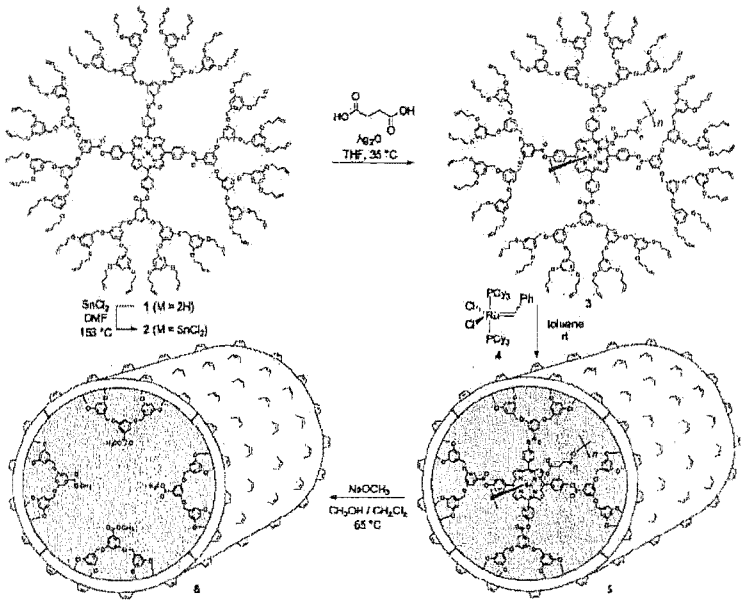
Scheme 1.14.

Grubbs *et al.* have recently used RCM in the preparation of rotaxanes, compounds composed of a dumbbell-shaped component around which one or more macrocycles are trapped (Scheme 1.15).[71] Compounds of this type have applications as molecular switches in the emerging field of molecular computing.



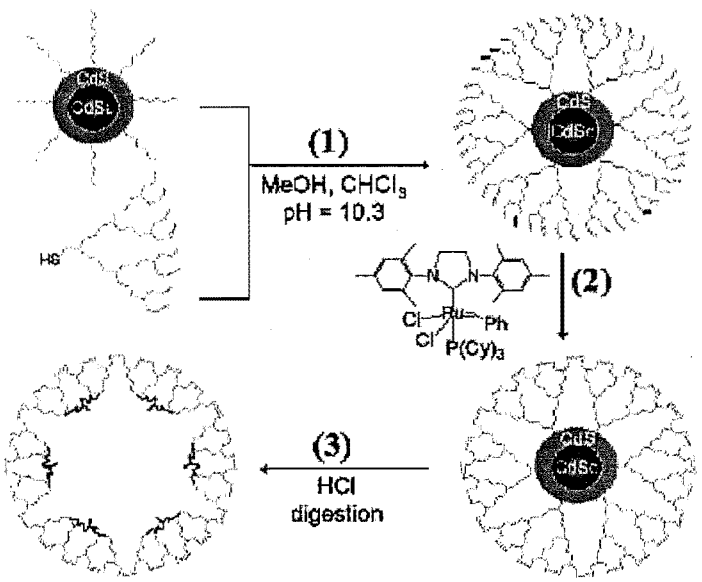
Scheme 1.15.

Novel uses of RCM in the field of dendrimer chemistry have led to a number of interesting applications. Zimmerman *et al.* used **1.40** in the controlled synthesis of discrete nanotubes (Scheme 1.16).[72]



Scheme 1.16.[72]

Peng *et al.*[73] used **1.42** to ring-close G3 surface dendrons during the preparation of luminescent semiconducting nanocrystals with enhanced chemical, photochemical, and thermal properties (Scheme 1.17).



Scheme 1.17.[73]

Currently, applications of olefin metathesis in the petrochemical industry include its use in the Phillips triolefin process to produce polymerisation-grade propene by cross-metathesis of ethane and 2-butene. This process has also been used in the production of neohexene, an intermediate in the synthesis of musk perfume. A large scale industrial process incorporating olefin metathesis is the Shell Higher Olefins Process (SHOP), in which ethene is converted to detergent range alkenes (C_{12}-C_{14}).

Olefin metathesis has also been applied to the synthesis of high tensile polymers. Grubbs ruthenium catalyst allows polymerisation to occur in the presence of fillers, additives, stabilizers and other ingredients in a polymer formulation, leading to stronger, more easily moldable polymer formulations An impressive example is the preparation of a 1.5 inch-thick polycyclopentadiene resin, prepared with ruthenium technology, that is impenetrable to 9mm bullets (Figure 1.22).

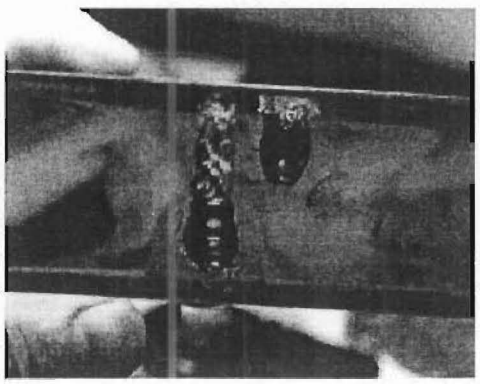
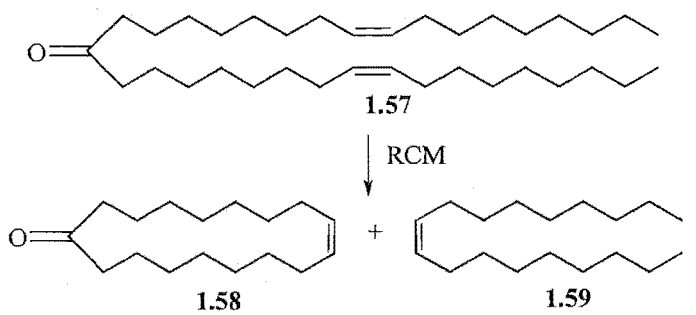


Figure 1.22.[74]

In an age where the availability of fossil fuelsis gradually decreasing and the demand for new sources of renewable feedstocks is increasing, olefin metathesis has seen application in the area of oleochemicals. Oleochemicals, incorporating natural fats and oils, have emerged as a distinctly viable and cost-effective source of raw materials and energy in this regard. Unlike petrochemicals, oleochemicals are derived from renewable resources, have good biostability, and no net CO_2 production. Natural oils and fats (composed predominantly of glyceryl esters of fatty acids) are used as starting materials in a wide range of chemical products. The most important are the long-chain vegetable oils such as soybean and rapeseed oil (unsaturated C18 fats), and palm oil (unsaturated C_{16} and C_{18}

fats), and the short and medium length vegetable oils such as coconut and palm kernel oil (C_{12}-C_{14}) that serve as important sources for the production of cosmetics, detergents, soaps and emulsifiers. One example in this area is the highly efficient conversion of oleon **1.57**, by RCM to a *cis-trans* mixture of **1.58** and 9-octadecene **1.59**, and (Scheme 1.18). The *cis* isomer of **1.58**, civetine, is an attractive ingredient in musk perfumes and is ordinarily prepared synthetically in poor yields over many steps.



Scheme 1.18.

Another example is the metathesis of fatty oils that contain triglycerides of unsaturated long-chain fatty acids, to form both intramolecular and intermolecular products. This is of particular interest in the printing industry. Thus, olive oil, consisting mainly of glycerol trioleate (triolien), yields 9-octadecene and polymeric triglycerides (Figure 1.19).

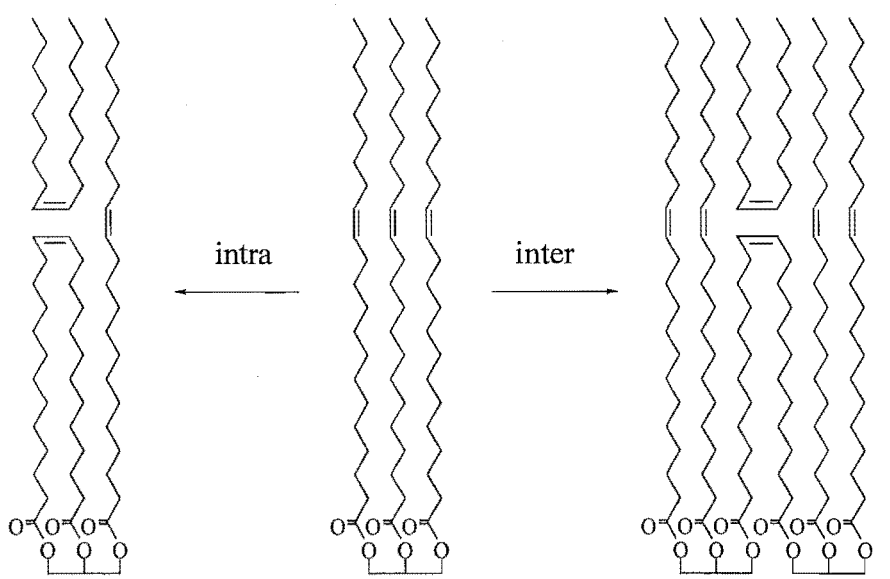


Figure 1.19.

Drying and semidrying oils such as linseed oil and soybean oil are valuable raw materials for the manufacture of oil-based paint, printing ink and synthetic resins. Metathesis of these oils, results in high molecular-weight oils (stand oils) that retain the integrity of their unsaturated double bonds. Such oils have more pronounced drying properties than thermally polymerised oils, where the polymerisation process considerably reduces the number of double bonds available for cross-linking during the drying process. Thus metathesized soybean oil has been used as an additive in low concentrations to benefit the printing process by dramatically reducing the drying time of soybean oil.

Other products that can be produced from RCM of oleochemicals include 1-triacontanol, a plant growth stimulant, insect pheromones, three of which are commercially available by means of olefin-metathesis-based syntheses, and various polymers. Reactions of this type offer increased efficiency over current processes due to low energy requirements, low accident potential due to mild reaction conditions, and the fact that all the metathesis products are useful. Many other commercial applications of olefin metathesis are currently being explored.

The future of ring-closing metathesis, and olefin metathesis in general, remains bright as new catalysts and applications are continually discovered.

1.5. Research Described in this Thesis

Peptidomimetics have found widespread application as biostable, bioavailable, and often potent mimetics of natural peptides. This thesis describes the application of ring-closing metathesis (RCM) to the synthesis of conformationally constrained peptide mimics derived from α- and β-amino acids. The ability to introduce substituents stereoselectively at the α-position of these mimics will also be discussed.

Chapter Two of this thesis describes the enantioselective synthesis of a class of conformationally constrained, α-substituted tetrahydropyridine and piperidine peptide

mimics, via RCM. The solid-state conformation of these mimics was examined and their application as potential *cis*-amide bond mimics discussed. The enantiomeric purity of a key intermediate was also analysed to determine the stereoselectivity of a key alkylation step, and assess the overall enantiomeric integrity of the synthesis.

Chapter Three describes the enantioselective synthesis of a class of conformationally constrained, α-substituted dehydroproline and proline peptide mimics, by RCM, in a manner similar to that detailed in Chapter Two. The solid-state conformation of a dehydroproline mimic was determined by X-ray crystal analysis and discussed in comparison with the conformations observed for the tetrahydropyridine and piperidine mimics described in Chapter Two.

Chapter Four describes the first enantioselective synthesis of the tetrahydropyridazinone core of a 2-oxo-1, 6-diazobicyclo(4,3,0)nonane-9-carboxylate β-strand template. Conformationally constrained bicyclic templates of this type have been designed as key components in inhibitors of serine proteases such as thrombin.

Chapter Five describes a versatile ring-closing metathesis (RCM) approach to the synthesis of aminocyclohexenylcarboxylic acids (ACHC's) that allows the preparation of derivatives that are either unsubstituted, or substituted at the α-position. This second class represents an important addition to this family of compounds.

Chapter Six describes a convenient and versatile ring-closing metathesis approach to the synthesis of aminocyclopentenylcarboxylic acids (ACPC's) from *L*-methionine. This approach allows for the preparation of derivatives that are either unsubstituted, or substituted, at the α-position in a manner similar to that detailed in Chapter Five.

Chapter Seven describes the a ring-closing metathesis approach towards the synthesis of six- and seven-membered cyclic lactams derived from functionalised α- or β-amino acids. This approach allows the preparation of derivatives that are either unsubstituted or substituted at the α-position.

1.6 References for Chapter One

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CHAPTER TWO

SYNTHESIS OF α-SUBSTITUTED TETRAHYDROPYRIDINE AND PIPERIDINE-BASED PEPTIDE MIMICS VIA RING–CLOSING METATHESIS

2.1 Introduction

In recent decades, the design and synthesis of peptidomimetics has become increasingly important, as researchers seek to understand a myriad of biological processes and search for new and more effective treatments of disease. Here we present the design, synthesis, and derivatisation of a new class of α-substituted tetrapiperidine-based peptide mimics, for use in this process

Piperidine and tetrahydropyridine ring systems are found widely in nature as components of biologically active natural products. Some simple examples are (+)-coniine, the major alkaloid component of poison hemlock, and lobeline, a component of *lobelia*, used by North American Indians as a substitute for tobacco. More complex multicyclic examples include (+)-cytisine and (+)-lupanine, the toxic quinolizidine-based lupin alkaloids found in species of *lupinus* (Leguminosae) (Figure 2.1).

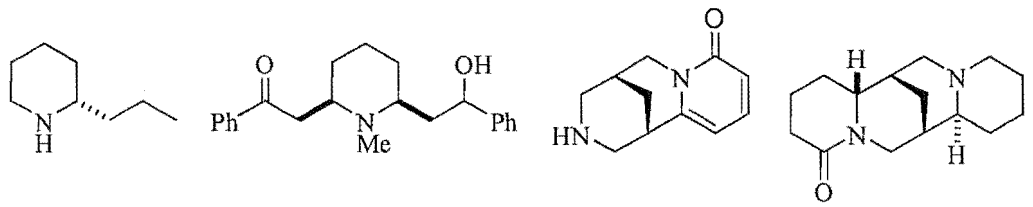


Figure 2.1. Examples of piperidine based natural products

An important derivative of piperidine, and a key intermediate in the biosynthesis of many important natural products, is *L*-pipecolic acid. This cyclic amino acid is produced via the lysine biosynthetic pathway. Here, the ε-amino group of *L*-lysine undergoes oxidative deamination, followed by Schiff base formation, to give $\Delta^{1,6}$-piperideine carboxylic acid, a key precursor to *L*-pipecolic acid (Figure 2.2). Critical to this pathway is the retention of the carboxylic acid group at Cα.

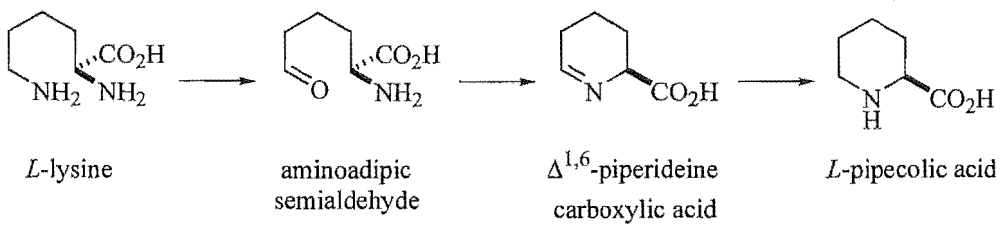


Figure 2.2. *L*-lysine derived biosynthesis of *L*-pipecolic acid

Many important classes of natural product are derived from this proline homologue e.g. swainsonine and castanospermine, members of a class of polyhydroxylated indolizidines isolated from the Moreton Bay chestnut *Castanospermum australe* (Leguminosae) (Figure 2.3). These compounds are active against the HIV retrovirus due to their ability to inhibit glycosidase enzymes involved in glycoprotein biosynthesis.

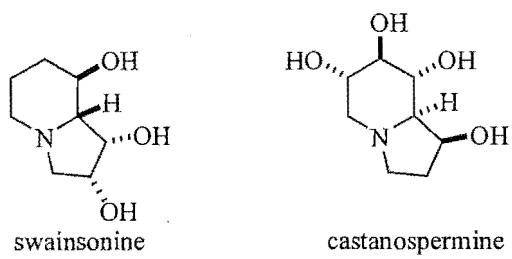


Figure 2.3. Examples of hydroxylated piperidine containing natural products.

L-Pipecolic acid is also the precursor to quinolinic and nicotinic acids, important components of many vitamins and coenzymes, including NAD^+ and $NADP^+$. Nicotine, anabasine and anatabine, the alkaloids found in tobacco (*Nicotiana tabacum* (Solanaceae)) are derived from these acids, as is the tetrahydro-derivative arecoline (Figure 2.4).

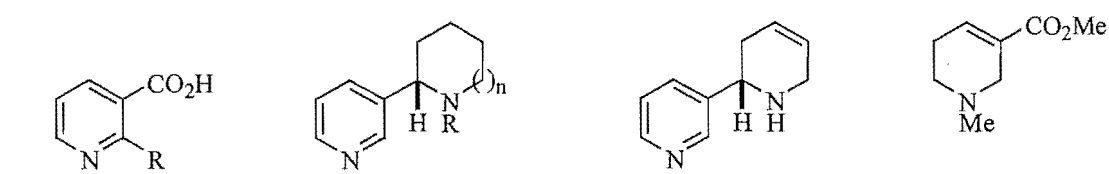
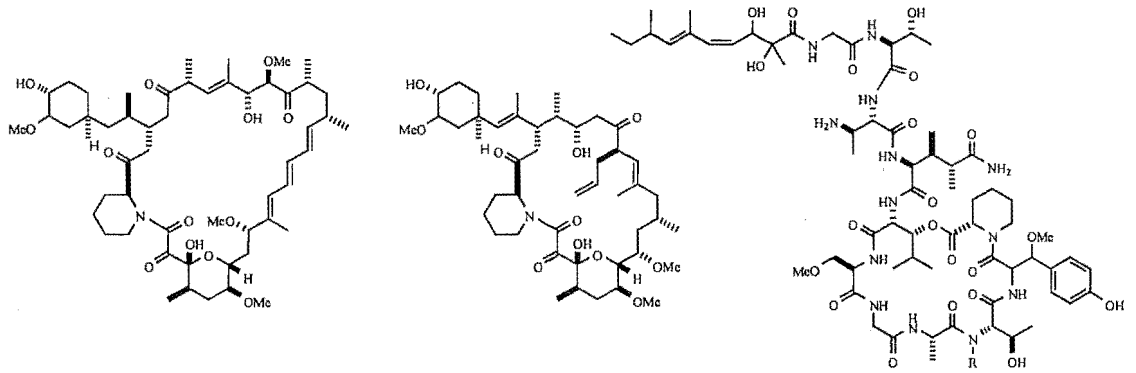


Figure 2.4. Many alkaloids and important vitamins and coenzymes are derived from *L*-pipecolic acid.

L-pipecolic acid can be found in a variety of biologically active natural peptides. Like proline, *L*-pipecolic brings a high degree of constraint about the amide bond acid when acylated at nitrogen. For proline, this results in important biological scaffolds such as *cis* amides and β-turns. Since proline is found in a wide range of structural and biologically active natural products it is not surprising to find compounds in which *L*-pipecolic acid serves a similar role. Some prominent examples of this are the macrolides FK-506 and rapamycin, and the cyclic depsipeptides papuamides A and B (Figure 2.5). FK-506 (tacrolimus), isolated from *Streptomyces tsukubaensis*, and rapamycin, isolated from *Streptomyces hygroscopius*, are immunosuppressants that are universally used in organ transplant surgery. Papuamides A and B,[1] isolated from the marine sponge *Theonella* collected in Papua New Guinea, are known to strongly inhibit HIV-1_{RF} infection of human T-lymphoblastanoid cells, and also exhibit potent cytotoxicity against a number of human cancer cell lines. This novel class of compound contains a pipecolate residue adjacent to a key, β-OMeTyr residue that is required for activity.

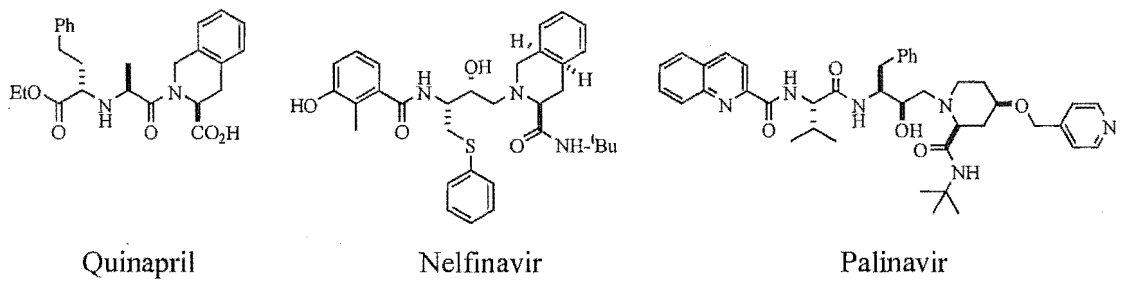


rapamycin tacrolimus (FK-506) Papuamide A R=Me B R=H

Figure 2.5. Examples of natural products containing an *L*-pipecolic acid subunit.

Given the occurrence of pipecolic acids in nature, it is not surprising that they have also been incorporated into a wide range of pharmaceuticals. Several synthetic peptidomimetics contain pipecolic acid subunits. For example, the potent ACE inhibitor quinapril, and the prominent HIV protease inhibitor Nelfinavir, used in AIDS therapies, both have constituent pipecolic acids that serve to stabilise the bioactive conformation of a

key amide bond. Palinavir, another highly potent inhibitor of HIV, incorporates a 4-hydroxy-pipecolic acid subunit as part of its structure (Figure 2.6).[2]

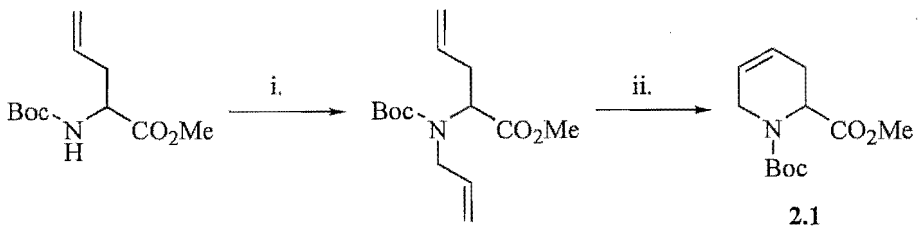


Quinapril Nelfinavir Palinavir

Figure 2.6. Substituted pipicolic acids are found in many important pharmaceuticals.

Substituted pipecolic acids have thus become the subject of intensive synthetic efforts in recent years. In addition to several racemic preparations of compounds of this type,[3,4,5] a number of asymmetric syntheses, from chiral building blocks, have also been reported (carbohydrates,[6,7] amino acids[8-12]).

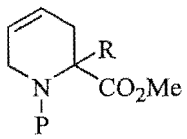
Another important compound related to pipecolic acid is the dehydro analog, i.e. 4,5-dehydropiperidine carboxylic acid **2.1** (baikiain). This olefin-containing cyclic amino was first isolated in 1950 from *Baikiaea plurijuga* (Rhodesian teak).[13] However, there have been surprisingly few syntheses described up till now.[14,15] One recent report describes the ring-closing metathesis cyclisation of an allylglycine derivative, to give N-Boc baikiain methyl ester **2.1**, in excellent yield (Scheme 2.1).[16] Several asymmetric syntheses have subsequently been developed utilising this methodolgy.[17,18]



2.1

Scheme 2.1. Grubbs' synthesis of *N*-Boc-(+/-)-tetrahydropipecolic acid methyl ester. *Reagents and conditions*: i. NaH, allyl bromide, DMF, 0° C for 1.5 h then rt for 30 min, 55%; ii. $RuCl_2(PCy_3)_2$=CHPh (**1.40**), benzene, rt, 2 h, 91%.

However, there are few reported examples of these compounds that contain an α-substituent. As such, we set out to develop a methodology whereby a substituent could be introduced stereoselectively at the α-carbon. This would allow access to conformationally restricted amino acid analogues of type **2.2**.



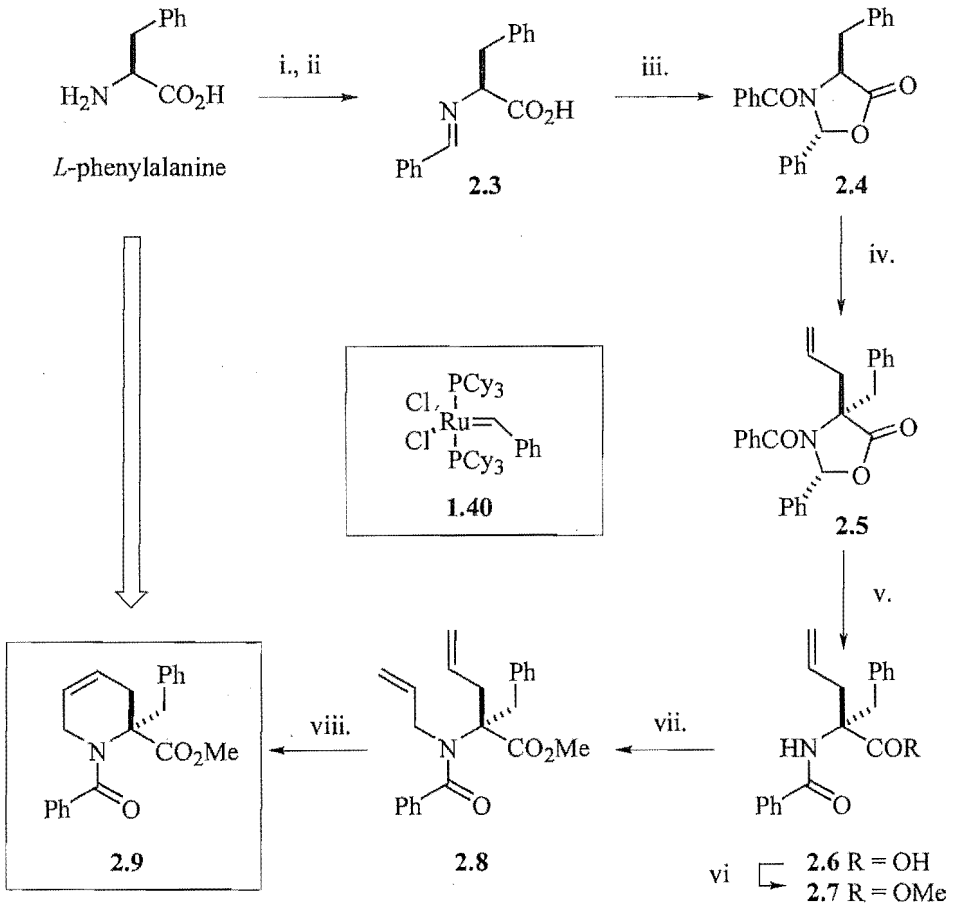
2.2

The introduction of a substituent at the α-carbon would allow interactions usually associated with amino acid side-chains, i.e hydrogen bonding, π-π-stacking, hydrophobicity, hydrophilicity etc, to be incorporated into the mimic, to give a compound with a unique conformational constraint and potential binding specificity. A general synthetic methodology would allow the incorporation of a range of R groups at the α-carbon, with stereochemical control being maintained throughout the synthesis. In this way a library of compounds of this type could be built up for use in peptidomimetic design.

We chose to use oxazolidinone chemistry pioneered by Seebach *et al*[19,20] to introduce various substituents into a suitable precursor for ring-closing metathesis.[16] The inclusion of an olefinic moiety allows for subsequent functionalisation at this position.

2.2 Synthesis of the Tetrahydropyridine Mimic

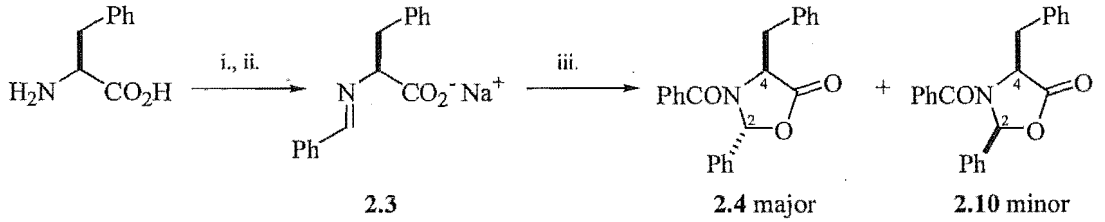
In the first instance we chose to introduce a benzyl group stereoselectively at the α-position of the proposed mimic (see **2.2** R=CH_2Ph), to give a phenylalanine analogue. This was chosen, as a model, for the introduction of an aromatic group to explore its effect on the properties of the mimic i.e π-π-stacking, hydrophobicity, should it be incorporated into a peptide. The proposed synthesis of this tetrahydropyridine mimic **2.9** is outlined in Scheme 2.2.



Scheme 2.2. *Reagents and Conditions*: i. NaOH; ii. PhCHO, CH_2Cl_2, reflux; iii. PhCOCl, CH_2Cl_2, 0° C for 4 h then rt for 16 h; iv. LiHMDS, THF, -78° C then allyl bromide; v. NaOH, MeOH, reflux; vi. CH_2N_2; vii. NaH, DMF, 0° C then allyl bromide; viii. Grubbs' ruthenium catalyst **1.40**, CH_2Cl_2.

Here, *L*-phenylalanine is protected as the diastereomerically pure *trans*-5-oxazolidinone **2.4**. This is then subjected to a stereoselective alkylation, with allyl bromide, to give **2.5**, which is subsequently hydrolysed, and alkylated on nitrogen, to give diene **2.8**. Ring-closing metathesis is then used to obtain the desired phenylalanine-derived tetrahydropyridine **2.9**.

The synthesis began with the preparation, from *L*-phenylalanine, of the key *trans* (or *anti*) oxazolidinone **2.4** (Scheme 2.3), a compound that has previously been reported.[20-22]



2.3 **2.4** major **2.10** minor

Scheme 2.3. *Reagents and Conditions*: i. NaOH; ii. PhCHO, CH_2Cl_2, reflux; iii. PhCOCl, CH_2Cl_2, 0° C for 4 h then rt for 16 h.

Using the method of Seebach and Fadel,[23] the sodium salt of *L*-phenylalanine was condensed, with azeotropic removal of water, with benzaldehyde in dichloromethane to give the corresponding Schiff base **2.3**. Acylation with benzoyl chloride, at 0°C for 4 h, followed by stirring at room temperature for 16 h, led to a 4:1 *trans* / *cis* diastereomeric mixture of oxazolidinones **2.4** and **2.10**. The diastereomers were separated by crystallisation from methanol to give the desired *trans* isomer **2.4** in 59% overall yield.[a,b]

The assignment of a *trans* (or *anti*) configuration to the major isomer **2.4** was confirmed by comparison with literature NMR data and further supported by the observation of a nuclear Overhauser enhancement (NOE) between **H2** and CH_2Ph.[22] In addition, a ^1H NMR spectrum of the *trans*-5-oxazolidinone **2.4**, at 23°C, revealed broadening of the resonances for the H2 proton, the benzyl CH_2 protons and a number of aromatic protons. It was suggested this might be due to conformational mobility of the molecule in solution. This conformational mobility could result from the partial double bond character exhibited by the amide bond of the ring nitrogen, thereby leading to restricted rotation (Figure 2.7).

[a] Crystallisation from ethyl acetate/petroleum ether gave 2.4 in comparable yield

[b] It is worth noting that literature methods for the synthesis of 5-oxazolidinones are consistently performed on a large scale, typically 20 - 40g. Research in this laboratory has found that synthesis on a smaller scale, 500mg - 5g, often gives yields somewhat lower than that reported for larger scales.

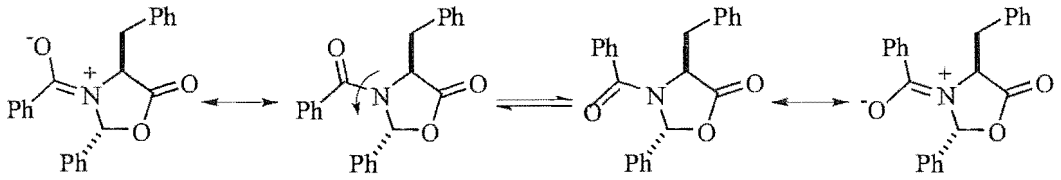
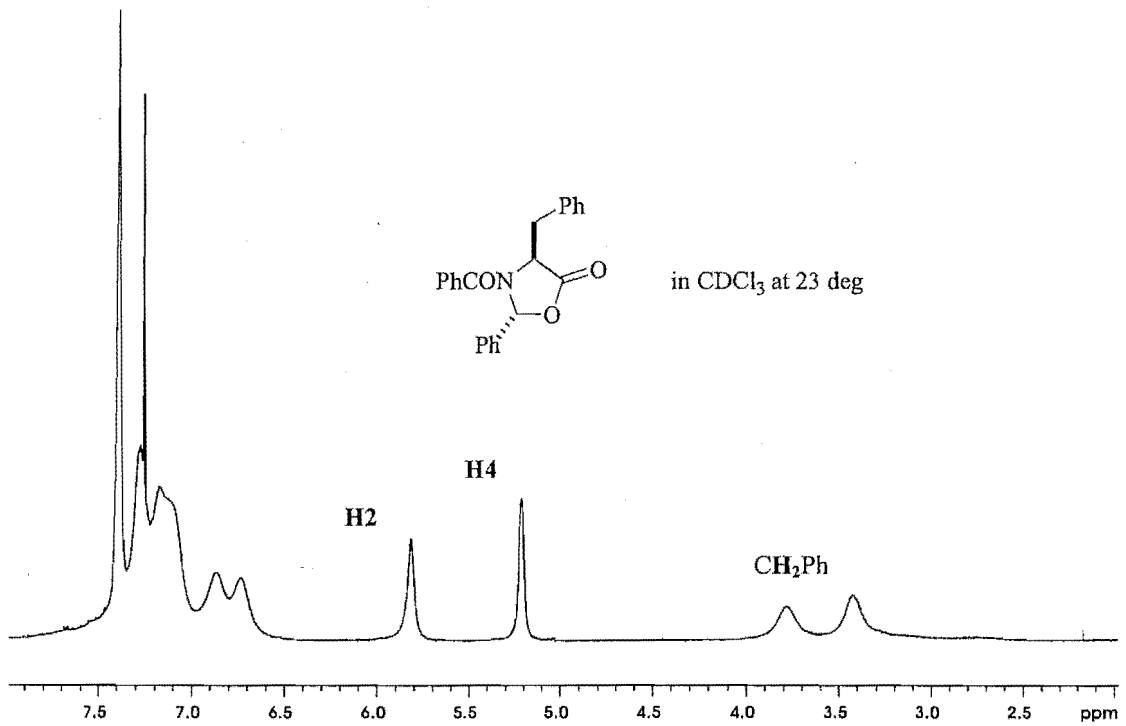


Figure 2.7. Broad ^{1}H NMR resonances for **2.4** may be due to conformational mobility in solution.

Figure 2.8 shows the ^{1}H NMR spectrum collected for the *trans* oxazolidinone **2.4**, in $CDCl_3$, at both 23° C and 50° C.



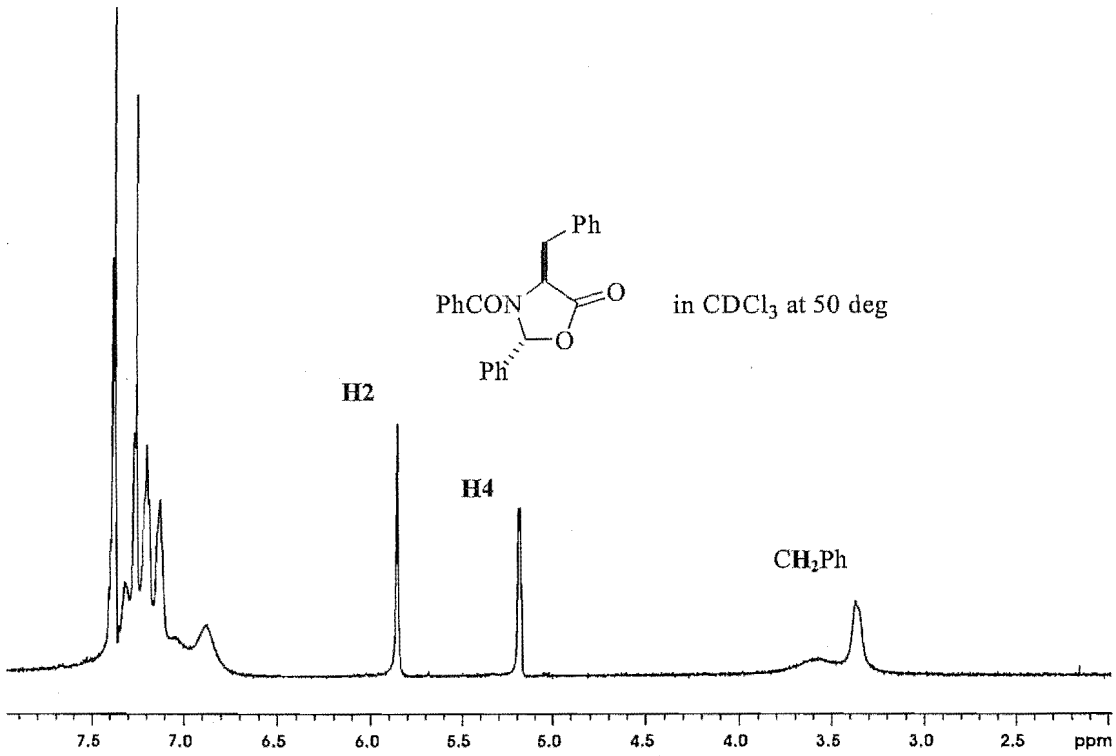
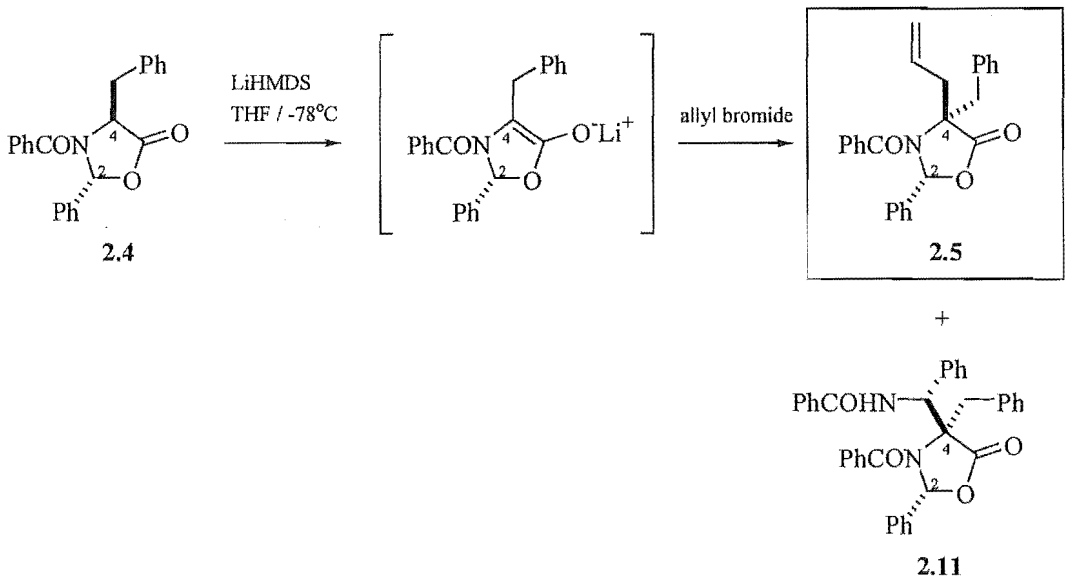


Figure 2.8. ^{1}H NMR spectra for the *trans* 5-oxazolidinone **2.4**, in $CDCl_3$, at 23° C and 50° C.

An increase in temperature resulted in a noticeable sharpening of the resonances for H2, H4, and some aromatic protons. This suggested that a coalescence of different conformations had occurred, a phenomenon that has been observed in numerous other 5-oxazolidinones, as well as other heterocycles.[24-26]

The introduction of the allyl substituent at C4 of **2.4** (refer Scheme 2.2), was next attempted. Oxazolidinone **2.4** was treated with LiHMDS, for 7 minutes at -78°C, in THF, to form an enolate. Allyl bromide was then added, and the mixture stirred at -78°C for 1 h, followed by warming to 20°C over 16 h. Pleasingly, this gave the desired alkylated 5-oxazolidinone **2.5**, in 93% yield after purification, as a single diastereoisomer by ^{1}H NMR (Scheme 2.4). A second compound, subsequently identified as the self-addition product **2.11** was also observed and isolated in 6% yield. It was found that short mixing times (5-7 minutes) between the addition of the base, and the addition of the electrophile, were needed to obtain high yields of **2.5**. Longer mixing times (20min-1h) resulted

predominantly in an excess of the self-addition products being isolated (see Section 2.5 Self Addition Products During Alkylation of 5-oxazolidinones).



Scheme 2.4. Alkylation of **2.4**, to give **2.5**, proceeds via a chiral enolate

The resulting configuration at C4 is defined by the absolute configuration at C2 of the starting oxazolidinone **2.4**, which is in turn governed by that of the starting amino acid. The substituent at C2 effectively blocks one face of the enolate derived from **2.4**, such that the electrophile approaches from the opposite side. It has been proposed that the enolate adopts a conformation in which the C2 phenyl group is pseudoequatorial, where the nitrogen and oxygen are pyramidal with the lone pairs pseudoaxial (Figure 2.9).[27] The combined steric effect of the phenyl group, and the *anti*-stereoelectronic effect of the 'oxonium' moiety, favours electrophilic attack *anti* to the phenyl group. This results in inversion of configuration at C4 of the alkylated product.

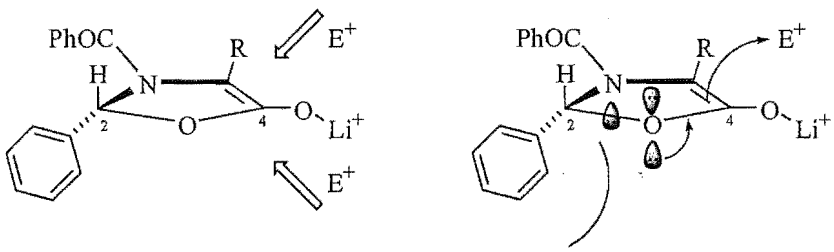


Figure 2.9. Alkylation of *trans*-5-oxazolidinones leads to an inversion of configuration at C4.

The configuration depicted in Scheme 2.4 was further supported by x-ray crystallographic analysis, with a perspective drawing of the solid-state structure of **2.5**, with atom labelling shown in Figure 2.10.

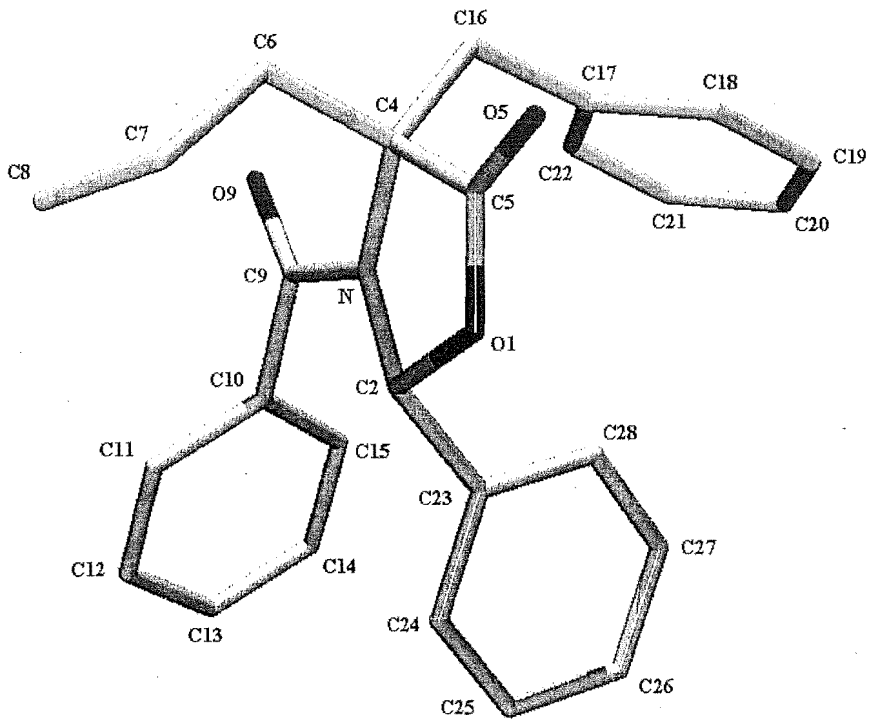
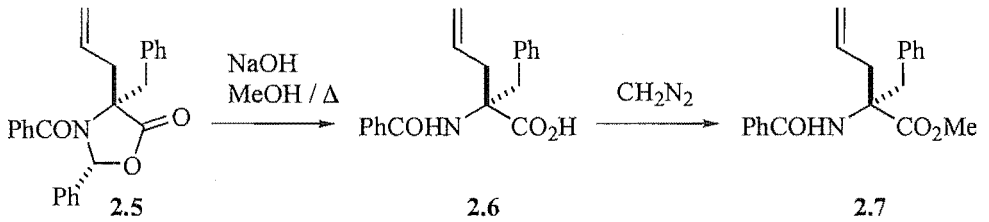


Figure 2.10. Solid-state structure of **2.5** with atom labelling.

Analysis of the solid-state structure of **2.5** allowed assignment of the relative stereochemistry as *trans*. The absolute configuration was assigned on the basis of the configuration of the starting amino acid, which determined the absolute stereochemistry at C2. In addition **2.5** was crystallised in the space group $P2_12_12_1$ with 4 super-imposable molecules in the unit cell, indicating that a chiral compound had been obtained. Measurement of the optical rotation for **2.5** gave an $[\alpha]_D = -2.9°$ (c=1.0, $CHCl_3$).

Hydrolysis of (-)-**2.5** with NaOH in methanol, followed by acidification, then gave the free acid **2.6**. Subsequent esterification, with diazomethane, gave the α,α-disubstituted amino

acid methyl ester **2.7**, in 91% overall yield over these two steps (Scheme 2.5, also refer Scheme 2.2).

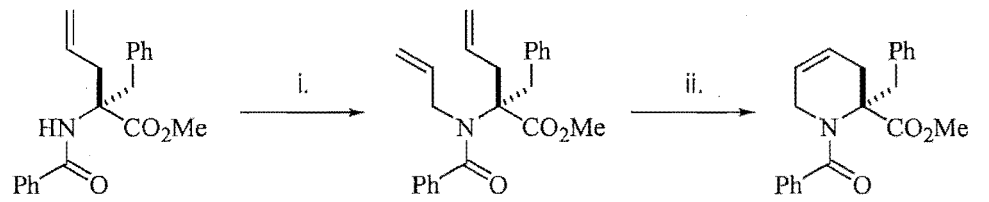


2.5 **2.6** **2.7**

Scheme 2.5. *Reagents and Conditions*: i. NaOH, MeOH, reflux, 1 h; ii. CH_2N_2, 0° C to r.t., 91%. Refer to Scheme 2.2.

Methyl ester **2.7** represents a key intermediate in this synthesis and has subsequently found use in other syntheses described later in this thesis (see Chapter 4 and 7).

The synthesis of the proposed tetrahydropyridine mimic **2.9** was then completed by first allylating the nitrogen of methyl ester **2.7**. Deprotonation of **2.7**, with sodium hydride in DMF, followed by treatment with allyl bromide, gave the dienic derivative **2.8**, which was isolated by silica chromatography in 30% yield.[c] Compound **2.8** was then cyclised at room temperature, with **1.40** using Grubbs' ruthenium alkylidine conditions,[16] to provide, after purification by flash chromatography, the doubly protected, phenylalanine-derived, 1,2,3,6-tetrahydropiperideine mimic **2.9** in 94% yield (Scheme 2.6).



2.7 **2.8** **2.9**

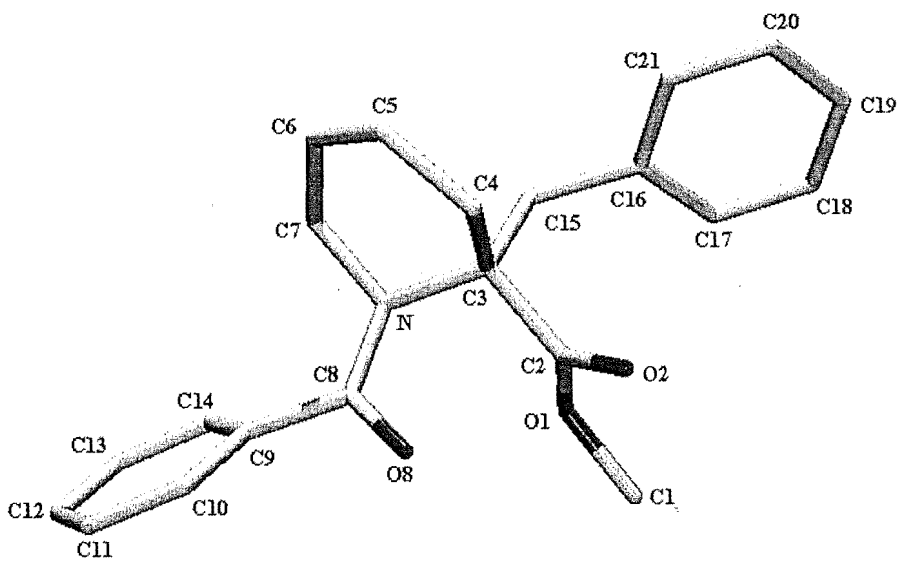
Scheme 2.6. Reagents and Conditions: i. NaH, allyl bromide, DMF, 0° C to rt, 30 %; ii. Grubbs' ruthenium catalyst A, CH_2Cl_2, rt, 94%.

[c] 23% of starting material was also recovered during purification and this was cycled back through the reaction to obtain more diene.

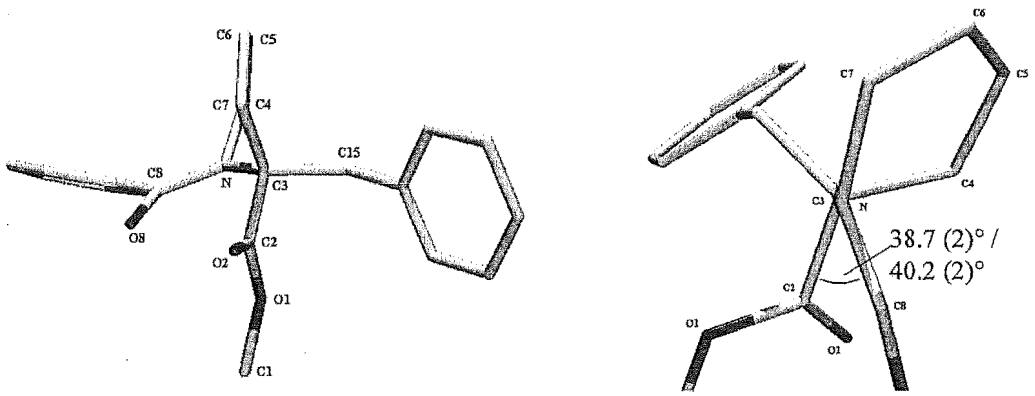
The factors that govern the ease of RCM reactions of peptide-based dienes are only now beginning to be explored.[28,29] Cyclisation of **2.8** occurred quickly and efficiently at room temperature, indicating that conditions were favourable for ring-closing metathesis. α,α-Disubstitution of an amino-acid-based diene substrate would also seem to favour the ease of RCM cyclisations, and is consistent with the Thorpe-Ingold, or gem-dimethyl effect. The present study, whereby **2.8** was readily cyclised to **2.9**, would appear to support this. Measurement of the optical rotation of a recrystallised sample of **2.9** gave an $[\alpha]_D$ = +38.2°, c=1.0 $CHCl_3$.

2.3 Solid State Conformation of the Tetrahydropyridine Mimic

Next, we desired to explore, and define, the conformational properties of the tetrahydropyridine peptide mimic **2.9**. Pleasingly, suitable crystals were obtained, from which the solid-state structure was determined, by X-ray crystallography, and satisfactorily refined. The asymmetric unit for compound **2.9** contained two independent molecules, which differed slightly in conformation, principally about the benzyl group. A perspective drawing of one of these molecules, with atom labelling, is presented in Figure 2.11.



a)



b) c)

Figure 2.11. a) Solid-state x-ray crystal structure of one conformer of the 1,2,3,6-tetrahydropyridine mimic **2.9**. b) View showing approximate planarity of the tetrahydropyridine ring (C4 - C7) with N and C3 deviating from the least squares plane. c) View along N-C3 axis indicating torsion angles about C8-N-C3-C2 peptide backbone are significantly shorter than that for proline.

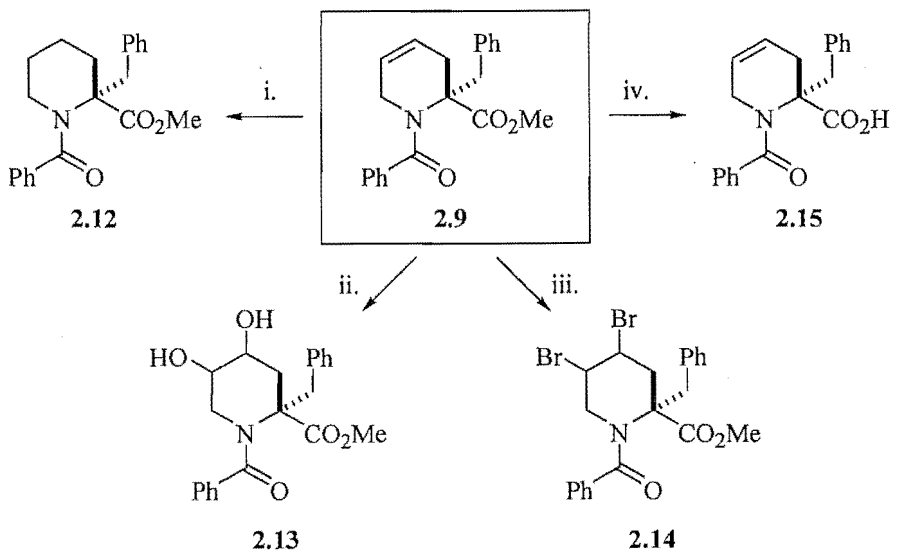
A proline-like N to Cα cyclization, as in **2.9**, results in significant restriction about the C8-N-C3-C2 peptide backbone torsion. This also narrows the conformational space explorable by the adjacent torsion angles. In the displayed crystal structure of **2.9** this peptide backbone torsion angle (torsion angles are given for both molecules in the asymmetric unit) is 38.7 (2)° / 40.2 (2)°, a value significantly shorter than that reported for proline.[30] The adjacent N-C3-C2-O1, C8-N-C3-C15 and C9-C8-N-C3 torsion angles are 53.24 (18)° / 50.60 (18)°, 155.51 (15)° / 157.20 (15)° and 175.97 (15)° / 177.90 (15)°, respectively. The magnitude of the C9-C8-N-C3 and C8-N-C3-C15 torsion angles are consistent with a Z amide bond and an *anti* relationship between the benzoyl and benzyl groups respectively. The C4, C5, C6 and C7 ring atoms are approximately in the same plane with N and C3 deviating from the least squares plane defined by the other four ring atoms by –0.2970 / -0.2739 Å and 0.4698 / 0.4904 Å, respectively. Some pyramidalisation of the amide nitrogen is evident with the angles at N summing to 351.14° / 350.17°.

In summary, solid-state x-ray analysis indicates that compound **2.9** adopts a *cis*-amide bond geometry, with significant conformational restriction occurring about the amide bond. This indicates that **2.9** possesses potential for use as both a proline mimic, as well as a β-turn mimic. Furthermore, 'fine tuning' of the conformation could be carried out via

the incorporation of various other substituents at the α-carbon. This could be used to explore, enhance, and maximise receptor site interactions should these compounds be incorporated into peptides for biological study

2.4 Derivatisation of the 1,2,3,6-Tetrahydropyridine Mimic

With the targeted cyclic mimic **2.9** in hand, and its conformational properties explored, we undertook a series of derivatisations. Efforts focussed on the functionalisation of the ring-bound olefin and the deprotection of the methyl ester to allow potential chain extension in the C direction. Scheme 2.7 depicts the derivatives synthesised from compound **2.9**.



Scheme 2.7. *Reagents and Conditions*: i. H_2, 10% Pd-on-C, EtOAc; ii. K_2OsO_4, NMO, H_2O / acetone; iii. Br_2, CH_2Cl_2; iv. NaOH, MeOH then H^+.

2.4.1 Preparation and Solid State Structure of an α-Substituted Piperidine Mimic

First, the saturated pipecolic acid analogue **2.12** was prepared via reduction of the ring-bound olefin. A sample of olefin **2.9** was dissolved in deoxygenated methanol, in the presence of palladium-on-carbon, and stirred for 16 h under a hydrogen atmosphere.

Filtration through Celite^{TM} and purification via radial silica chromatography gave compound **2.12** , in 92% yield, crystals of which gave an $[\alpha]_D$=+163.0, c=1.0 $CHCl_3$. Fortunately, recrystallisation of **2.12** yielded crystals suitable for X-ray crystallographic analysis. This was significant as it allowed us to explore and define the conformation of **2.12**, and make direct comparisons to its olefin containing precursor **2.9**. X-Ray analysis of **2.12** revealed it to have crystallised in the space group $P2_12_12_1$, with 4 super-imposable molecules in the unit cell. Perspective drawings of **2.12**, with atom labelling, are shown in Figure 2.12.

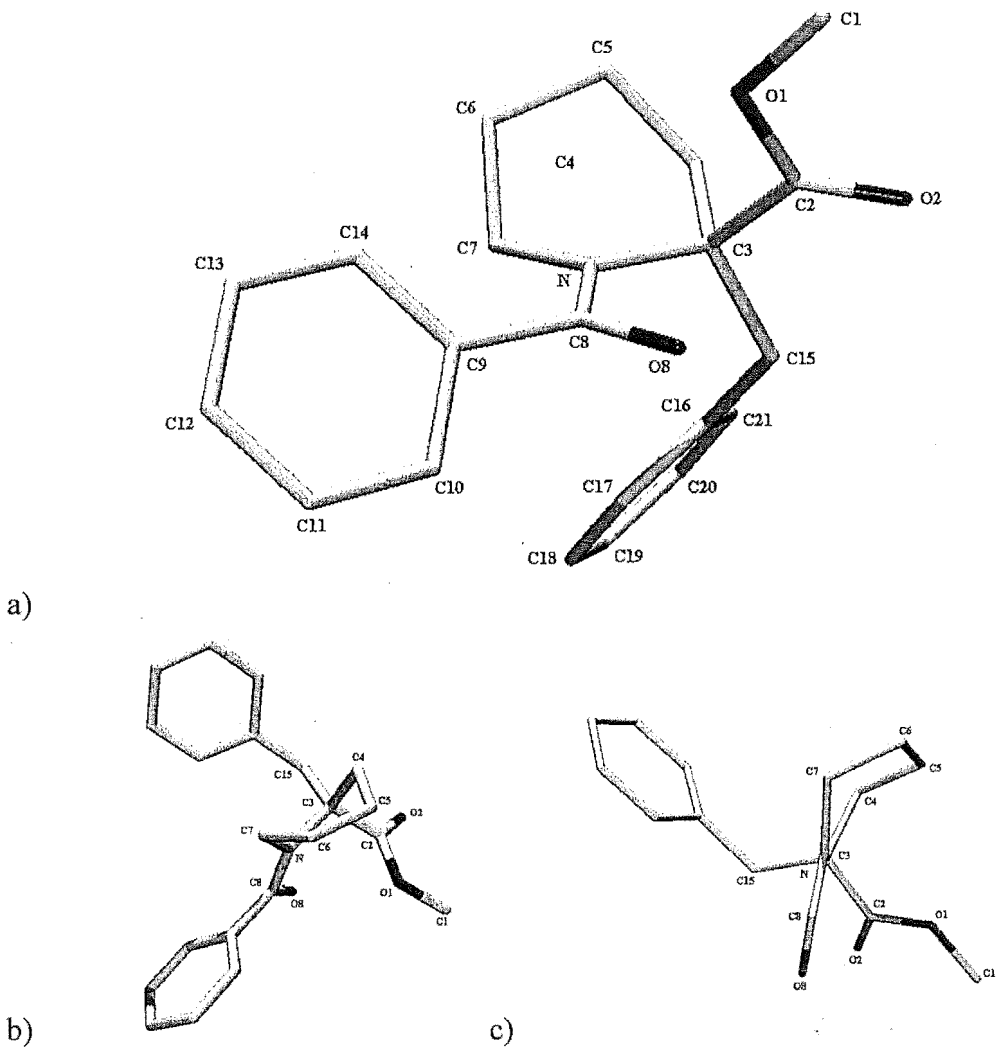


Figure 2.12. Solid-state X-ray crystal structure of the piperidine mimic **2.12**. b) View showing boat shaped conformation adopted by the piperidine ring. c) View along N-C3 axis indicating torsion angles about C8-N-C3-C2 peptide backbone.

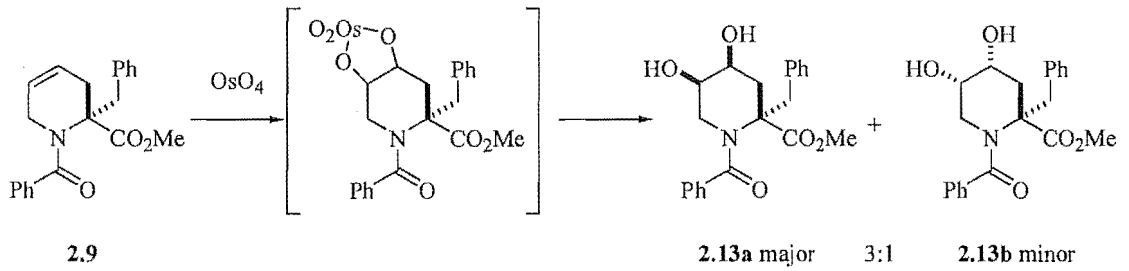
In the current structure of **2.12**, the N to Cα cyclization was again seen to impose significant restriction about the C8-N-C3-C2 peptide backbone. The torsion angle in this case was -47.7 (3)° compared with 38.7 (2)° / 40.2 (2)° for compound **2.9**. This amounts to a significant change in conformation, and corresponds to an almost 90° clockwise rotation about the amide bond when compared to **2.9**. The adjacent N-C3-C2-O1, C8-N-C3-C15 and C9-C8-N-C3 torsion angles are 40.0(3)°, 71.4 (3)° and 179.5 (2)°, respectively with the magnitude of the C9-C8-N-C3 and C8-N-C3-C15 torsion angles again consistent with a Z amide bond. As expected, the *anti* relationship between the benzoyl and benzyl groups was still evident, despite the change in conformation. The ring atoms N, C3-C7 adopt a distinct boat conformation with no significant pyramidalisation of the amide nitrogen evident (angles at N sum to 359.5 °).

In summary, solid-state x-ray analysis of compound **2.12** revealed significant conformational restriction about the amide bond, in a manner similar to that of **2.9**. It was also observed that the reduction of the olefin resulted in an inversion of torsion angle, i.e. a 'conformational flip', about the amide bond compared with that of compound **2.9**. As with **2.9**, **2.12** offers potential for use as both a proline mimic, and a β-turn mimic, with the difference in conformation between the two compounds providing a key contrast. Peptides incorporating these two motifs would adopt significantly different conformations. This highlights that the ability to control the conformation about an amide bond is critical in the design and synthesis of peptidomimetics..

Efforts were next directed toward derivatisation of the double bond via a *syn*-dihydroxylation, and a *trans*-dibromination. Dihydroxylation was chosen to give a compound analogous to the biologically important hydroxyproline, with the incorporation of polyhydroxylated subunits into peptides also known to lead to more water-soluble compounds. Dibromination, via the addition of bromine, was chosen for its classical use amongst olefin chemistry for giving *anti*-addition across a double bond. This was envisaged to allow a comparison between *cis*- and *trans*-substituted derivatives

2.4.2 Preparation of a Dihydroxypiperidine Analogue

There are many reagents that add OH groups to a double bond.[31] Osmium tetroxide (OsO_4)[32] was used as the reagent of choice in the dihydroxylation of **2.9**, for its known ability to give *syn* addition from the less hindered side of a double bond. Potassium osmate and *N*-methylmorpholine-*N*-oxide were therefore added to a solution of **2.9** in H_2O:acetone and stirred at room temperature for 24 h (Scheme 2.8).



2.9 **2.13a** major 3:1 **2.13b** minor

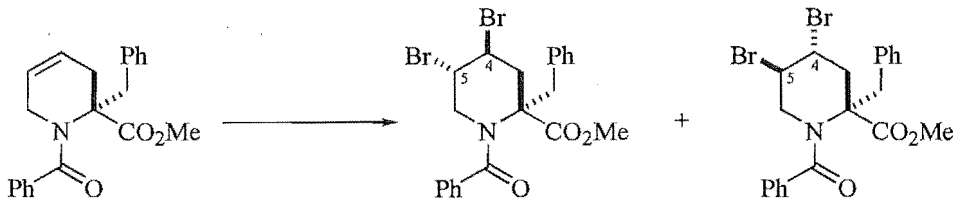
Scheme 2.8. *Reagents and Conditions*: K_2OsO_4, NMO, H_2O:acetone (8:3), rt 24 h, 81%.

The reaction yielded a mixture of dihydroxy compounds **2.13a** and **2.13b** in a ratio of 3:1, based upon integration of the ^{1}H NMR resonances for the corresponding methyl ester peaks. Silica chromatography allowed for the separation of a fraction containing the major isomer, which was isolated in 55% overall yield. Separation of the minor isomer proved more difficult, with chromatography leading to the isolation of a fraction containing a 1:2 mixture of the major and minor isomers, in a combined yield of 26%. Compound **2.13a** was tentatively assigned as the major isomer based on grounds that the tetroxide dianion (see Scheme 2.8) is favoured in its approach of the olefin, from the face opposite that of the benzyl group.

2.4.3 Preparation of a Dibromopiperidine Analogue

Dibromination was carried out by addition of bromine to a solution of **2.9**, in dichloromethane, until a permanent light brown colour was observed. The crude product was isolated in 68% yield, with ^{1}H NMR spectrum analysis revealing the presence of two

isomers, in a ratio of 4:1, based on a comparison of integrals for equivalent methyl ester resonances at δ_H 3.87 and 3.77ppm respectively. Purification by chromatography allowed isolation of the major isomer in 51% yield (Scheme 2.9). Analysis by NMR spectroscopy and mass spectrometry confirmed the presence of a single compound, with the ^{1}H NMR spectrum displaying a distinct methyl ester resonance at δ_H=3.87ppm.



2.9 **2.14a** major 4:1 **2.14b** minor

Scheme 2.9. Reagents and Conditions: Br_2, CH_2Cl_2, rt, **2.14a** 51%.

Compound **2.14a** was assigned as the major isomer (4*S*,5*S*), based on the grounds that addition of bromine, and formation of the bromonium ion, would predominantly occur on the least hindered face of the ring i.e. opposite that of the benzyl group. Subsequent attack with bromide could then proceed via two possible pathways. These two pathways are depicted in Figure 2.13. The solid-state structures of both **2.9** and **2.12** (see Figures 2.11 and 2.12) suggest that pathway B would be the minor pathway, as attack at C5 would be partially hindered by the benzyl group. Pathway A is less hindered in this regard and favours nucleophilic attack at C6. The result is formation of the major isomer **2.14a**.

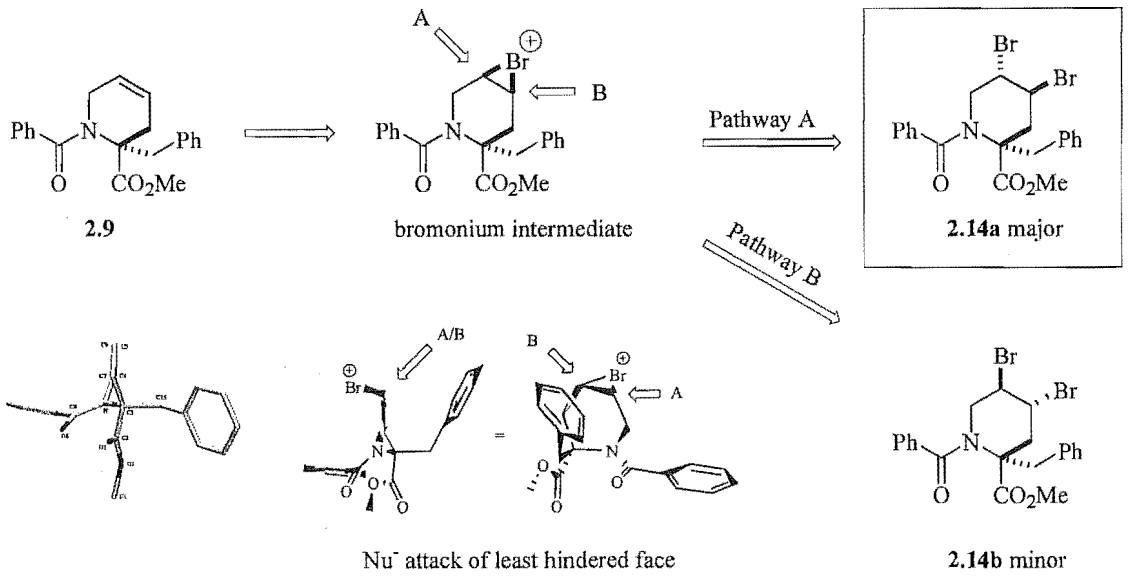
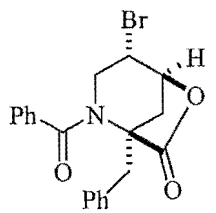


Figure 2.13. Additional of bromide to the bromonium intermediate can occur via two pathways. Pathway A, and formation of **2.14a**, is favoured due to the absence of steric effects associated with the benzyl group.

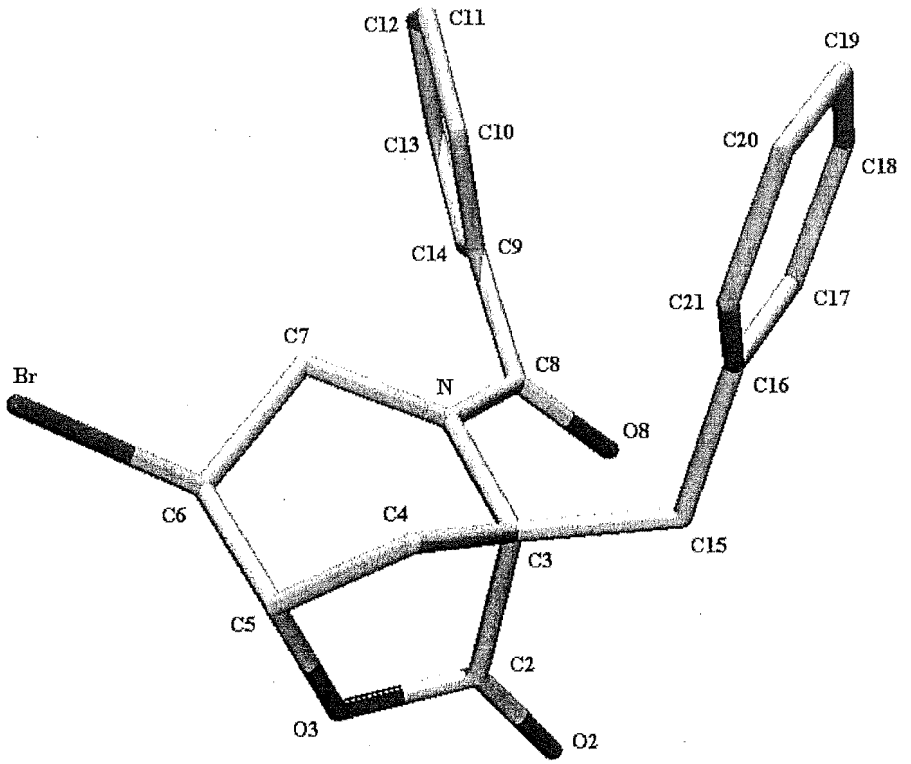
2.4.4 Solid State Structure of a Bicyclic Lactone Piperidine Analogue

We attempted to confirm the stereochemistry of **2.14a** (see Figure 2.13) by growing crystals of the major isomer, suitable for x-ray crystallography. This was unsuccessful in the short term and the sample was eventually set aside. However 6 months later a single crystal was discovered and found to be suitable for X-ray analysis. To our surprise, analysis of the crystal revealed the presence of the bicyclic compound **2.16**, rather than the expected dibromide **2.14a**. Since the ^{1}H NMR spectrum for **2.14a** clearly indicated the presence of a methyl ester (δ_H=3.87ppm), the indication was that this new compound, **2.16**, had been formed via lactonisation with displacement of bromine.

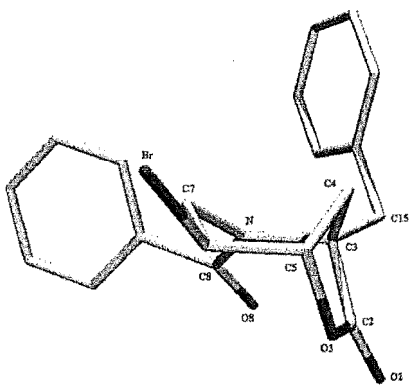


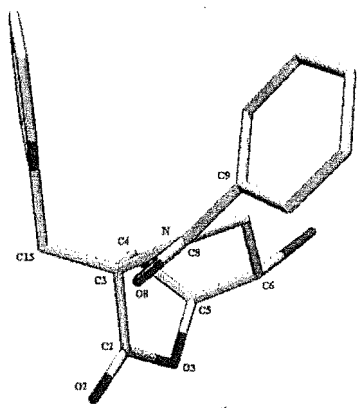
2.16

X-ray crystallographic analysis revealed **2.16** to have crystallised in the space group $P2_12_12_1$, with 4 super-imposable molecules in the unit cell. Perspective drawings of **2.16**, with atom labelling, are shown in Figure 2.14.



a)





b) c)

Figure 2.14. a) Solid-state x-ray crystal structure of **2.16**, with crystallographic numbering scheme b) View showing boat shaped conformation adopted by the tetrahydropyridine ring c) View along N-C8 axis indicating a slight twist about the amide bond.

It is apparent that the piperidine ring atoms, of **2.16**, represented by N, C3-C7 adopt a distinct boat conformation, with a five-membered lactone ring bridge existing between C5 and the carboxyl group extending from C3. A distorsion of normal tetrahedral geometry (bond angle is 107°) is observed about C3 due to the formation of this lactone. Bond angles around C3, internal to the ring system, for C2-C3-C4, C2-C3-N and C4-C3-N, are 100.3 (2)°, 106.8 (2)° and 106.7 (2)° respectively. Bond angles for C3, external to the ring system, for N-C3-C15, C2-C3-C15 and C4-C3-C15 are 115.0 (3)°, 110.7 (2)° and 116.0 (2)° respectively. The torsion angle about the peptide backbone of C8-N-C3-C2 is –78.9 (3)°. A slight twisting of the amide bond is also observed, with the magnitude of the torsion angle about O8-C8-N-C3 being 15.1 (4)°. The torsion angle about the amide bond represented by C9-C8-N-C3 is –159.9 (2)° indicating a *trans* amide bond relationship exists along the peptide backbone. No significant pyrimidalisation of the amide nitrogen is observed with the angles at N summing to 359.2°. The absolute stereochemistry about C6 was assigned as *S*, as Br and O3 adopt an *anti* relationship across the piperidine ring. This observation was significant as it confirmed the assignment of the absolute stereochemistry of the major dibromide isomer **2.14a** as (4*S*,5*S*), an assignment that had previously been based on steric grounds (see Scheme 2.9 and Figure 2.13). Unfortunately, the crystal of **2.16** was not recovered, and with the remaining sample having decomposed, no NMR data was obtained for characterisation and comparison with that of **2.12a**.

A proposed mechanism of formation of **2.16** is outlined in Figure 2.15. Ester hydrolysis of **2.14a**, followed by nucleophilic attack of the carboxylic acid group at C5 would result in loss of HBr to give lactone **2.16**. The presence of HBr would catalyse the hydrolysis of the ester. Lactone formation of this type is not without precedent, with halogen-mediated olefin functionalisation of *N*-protected baikiain and *N*-protected aminocyclohexene carboxylic acid having been used to access various hydroxylated derivatives.[33-35] However, lactonisation in this case occurs with retention of configuration at C4 suggesting an alternate mechanism may be possible.

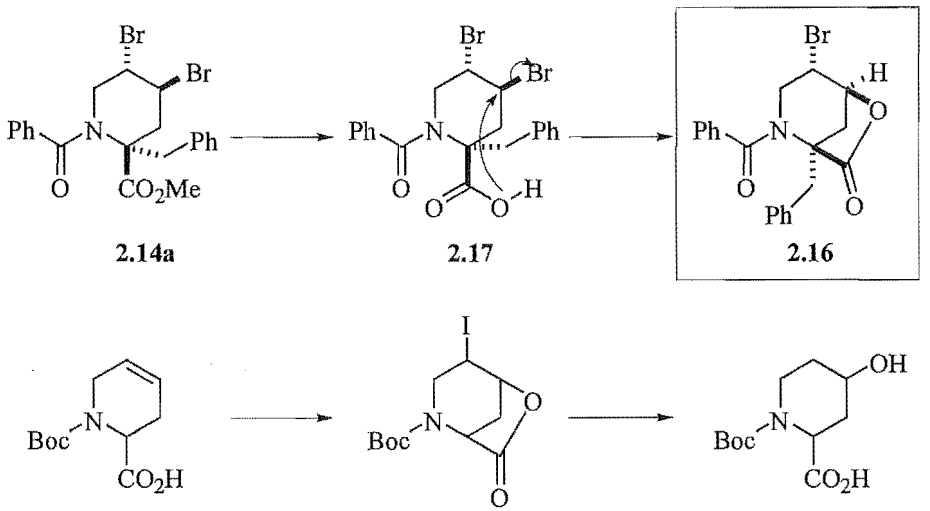
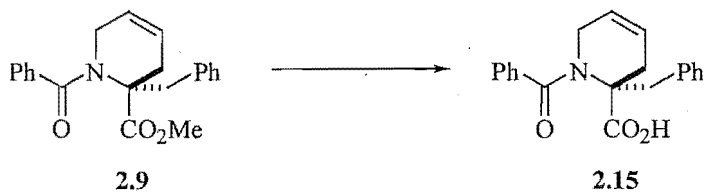


Figure 2.15. Proposed mechanism of formation of the bicyclic lactone **2.16**

2.4.5 Preparation of the Tetrahydropyridine Free Acid

Next, we desired to prepare a derivative suitable for incorporation of compound **2.9**, and its derivatives, into peptides via the carboxyl group. This was achieved via hydrolysis of the methyl ester to form the free acid **2.15** (Scheme 2.10).

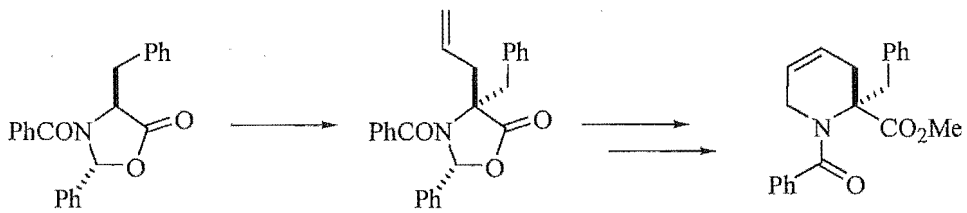


Scheme 2.10. *Reagents and Conditions*: NaOH, MeOH, reflux, 16 h, 92%.

Hydrolysis of a solution of methyl ester **2.9**, in methanol, with an excess of 1M aqueous NaOH at reflux overnight, gave the free acid **2.15** in 92% yield.

2.5 Self-Addition Products During the Alkylation of 5 Oxazolidinones

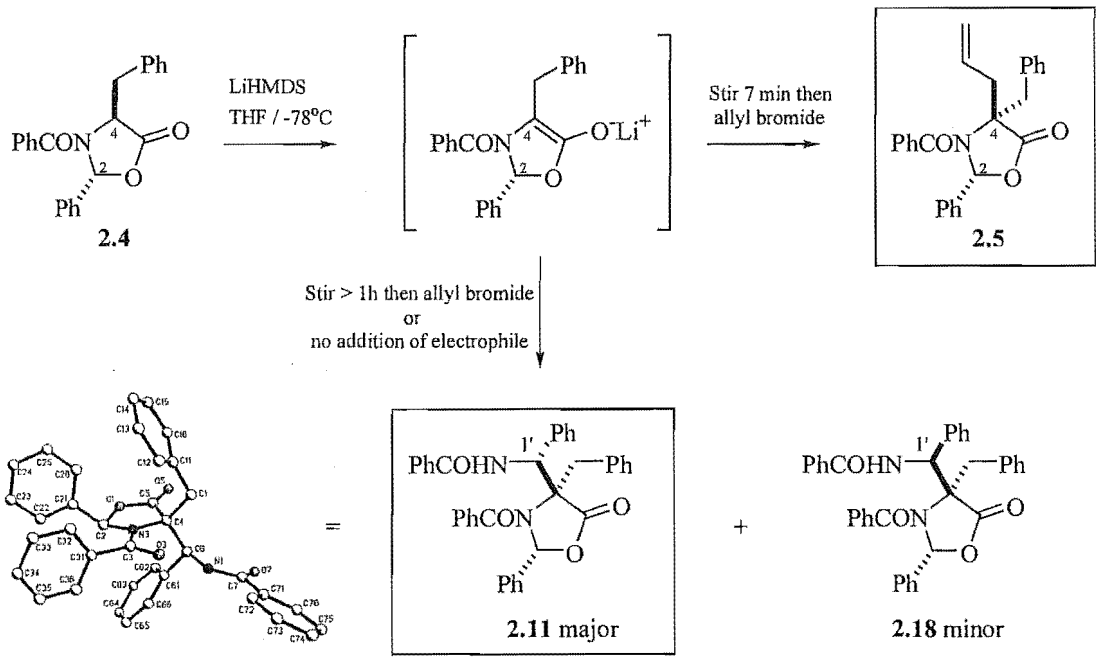
A key reaction in the overall synthesis of the tetrahydropyridine mimic **2.9** was the stereoselective alkylation of the benzyl-5-oxazolidinone **2.4** to give **2.5** (Figure 2.16, Scheme 2.2 and 2.4).



2.4 **2.5** **2.9**

Figure 2.16.

During the alkylation of **2.4**, to give **2.5**, the time between addition of the base (LiHMDS), and addition of the electrophile (allyl bromide), was found to be crucial. It was observed that a shorter mixing time (5-7 min.) was required to give the desired alkylated product **2.5**, while a longer mixing time (> 1 h) resulted in the excess formation of the self-addition compound **2.11** (Scheme 2.11). Intermediate mixing times (20-30 min) gave a mixture of desired alkylated product **2.5** and self-addition product **2.11**.



Scheme 2.11. Extended mixing times between addition of the base, and addition of the electrophile, resulted in the formation of self-addition products .

To further explore the formation of this self-addition product, **2.4** was dissolved in THF at -78° C, and treated with LiHMDS in the absence of an electrophile. The reaction was stirred at -78° C for 2h and was then allowed to warm to rt over 16h. Compound **2.11** was isolated from this experiment in 55% yield, with a ^{1}H NMR spectrum of the crude mixture revealing ~5% of a second diastereomer of **2.11**, **2.18**. This compound, which was not purified, was tentatively assigned as the C-1' epimer of **2.11** based on the downfield position of the 2-H resonance (δ_H 4.92) compared with **2.11** (δ_H 4.72). The stereochemical assignment of compound **2.11**, as depicted, has previously been deduced via X-ray crystal analysis.[22]

A possible mechanism for the formation of **2.11** would involve base-catalysed fragmentation of the precursor **2.4**, to give an N-acylimine of type **2.19**, which would then react with the oxazolidinone enolate at C4 (Figure 2.16). This fragmentation is analogous to the heterolytic cleavage of α-substituted amides to give N-acyliminium ions.

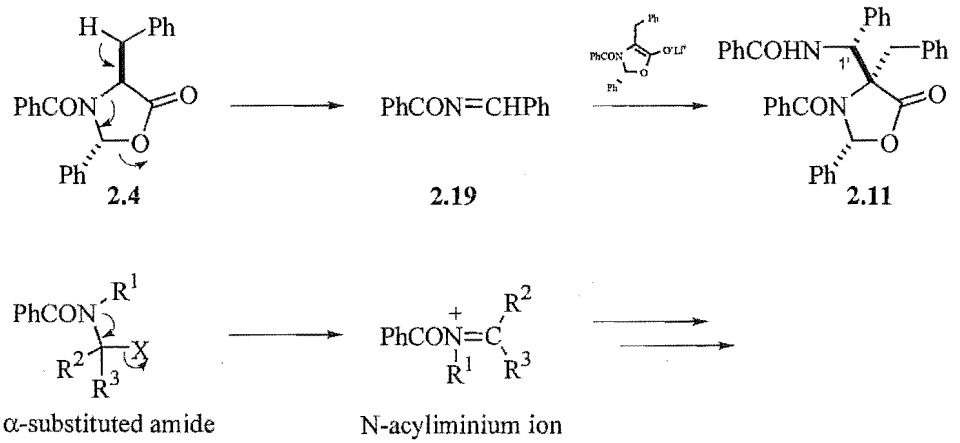


Figure 2.16. Proposed mechanism of formation of self-addition product **2.11**.

A proposed transition state for the reaction between the enolate derived from **2.4** and the acylimine **2.19,** is shown in Fig 2.17. From this it is apparent that reaction occurs from the side opposite the C2 phenyl group with *ul*-1,3-induction (Figure 2.16).[22]

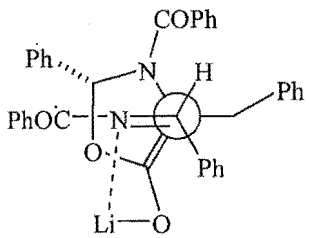
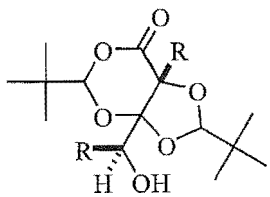


Figure 2.17. Reaction between the enolate derived from **2.4**, and the acylimine **2.19**, occurs with *ul*-1, 3-induction

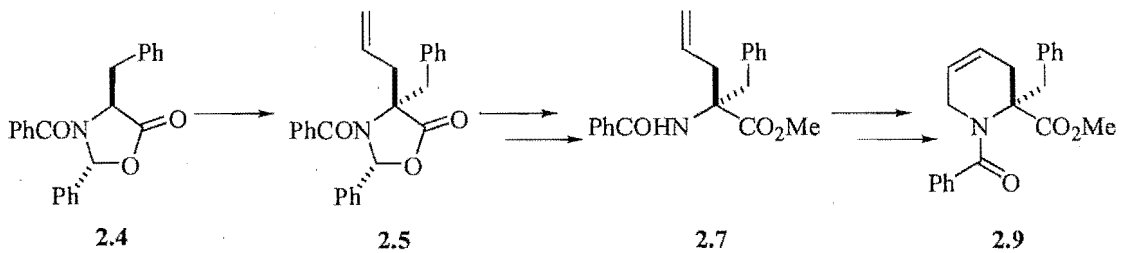
This results in the stereochemistry of **2.11**, depicted in Figure 2.16 and Scheme 2.11, occurring with >90% diastereoselectivity. Formation of other self-addition products in alkylations of 1,3-oxazolidinones,[36] and related 1,3-oxazolanones,[37] have also been reported. For example compounds **2.20a** and **2.20b** have been isolated from the alkylation of phenylalanine- and alanine-derived 2-(*tert*-butyl)-1, 3-dioxolanones.[37]



2.20a R=Me
2.20b R=CH_2Ph

2.6 Determination of the Enantiomeric Purity of a Key Intermediate

Synthesis of the tetrahydropyridine mimic **2.9**, outlined in Scheme 2.2, and abbreviated in Figure 2.18, proved successful, with the stereochemistry being established by X-ray crystallography.



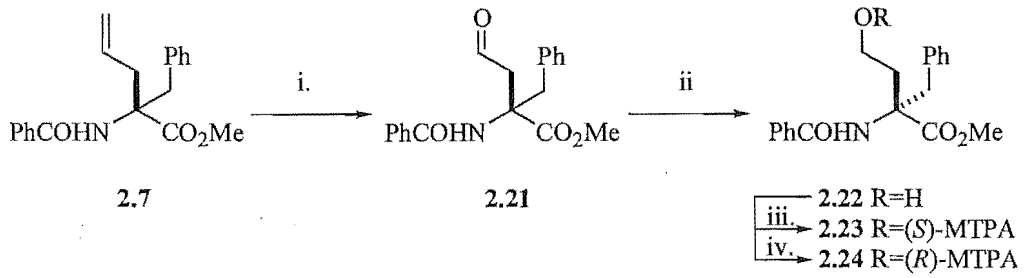
2.4 **2.5** **2.7** **2.9**

Figure 2.18. Synthesis of tetrahydropyridine mimic **2.9** from benzyl-5-oxazolidinone **2.4**.

The key to the enantioselective synthesis of **2.9** was the stereoselective alkylation of the benzyl 5-oxazolidinone **2.4**, with allyl bromide, to give the dialkylated 5-oxazolidinone **2.5**. Crystallisation of this compound, and analysis by ^1H NMR, revealed the absence of any minor diastereoisomer. Hydrolysis of **2.5**, followed by esterification, gave the key intermediate **2.7**, which was subsequently used in the synthesis of mimic **2.9**.

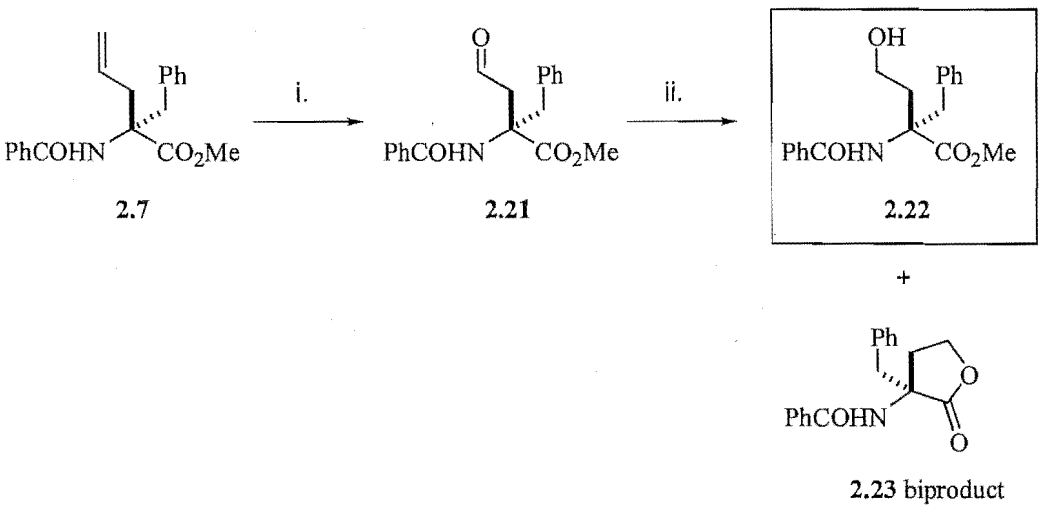
During the preparation of **2.4**, it was observed that, while crystallisation resulted in the absence of the minor diastereoisomer by ^1H NMR, the optical rotation ($[\alpha]_D$ = +267°, c=1.0, $CHCl_3$) did not correspond to that reported in the literature ($[\alpha]_D$ = +385.2, c=1.0, $CHCl_3$). To address this issue, and to confirm the overall stereochemical integrity of the synthesis, the enantiomeric purity of the key derivative **2.7** was determined. This was achieved by conversion of **2.7** to the corresponding (*R*)- and (*S*)-Mosher esters (Scheme

2.12), and subsequent examination of their crude 1H NMR spectra for evidence of any minor isomer.



Scheme 2.12. *Reagents and Conditions*: i. O_3, CH_2Cl_2 / MeOH (3:1), -78° C; ii. $LiBH_4$, CH_2Cl_2; iii. DMAP, Et_3N, (*S*)-MTPA-Cl, CH_2Cl_2, r.t., 5 min; iv. DMAP, Et_3N, (*R*)-MTPA-Cl, CH_2Cl_2, r.t., 5 min. (MTPA-Cl = methoxy-α-trifluorophenyl acetyl chloride)

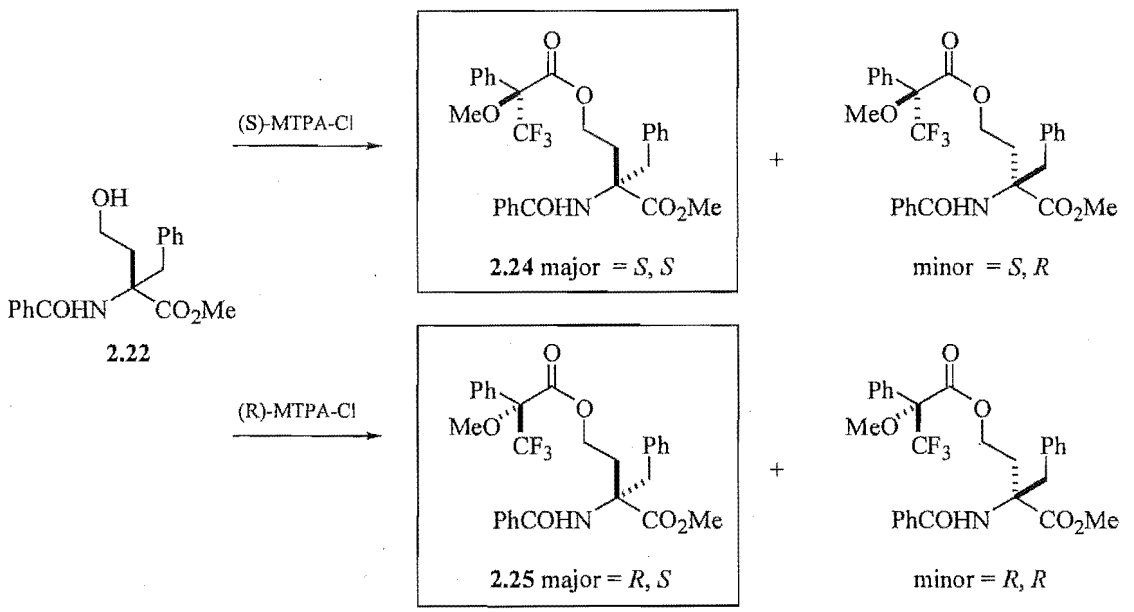
The alkene **2.7** was first converted to the corresponding alcohol **2.22**. Ozonolysis of **2.7**, in a 3:1 mixture of dichloromethane / methanol at -78°C, in the presence of solid sodium bicarbonate, gave aldehyde **2.21** in excellent yield (96%). Reduction with lithium borohydride resulted in a mixture of the desired alcohol **2.22** (41%), and the cyclic lactone by-product **2.23** (53%), in a ratio of 1:1.3 (Scheme 2.13).



Scheme 2.13. *Reagents and Conditions*: i. O_3, CH_2Cl_2 / MeOH (3:1), -78° C, 96%; ii. $LiBH_4$, CH_2Cl_2, 41% **2.22**, 53%, **2.23**.

Optimisation of this reaction, to avoid the formation of **2.23**, was not pursued as a sufficient quantity of **2.22** was obtained during the reduction. All products were isolated by chromatography thereby retaining any enantiomeric mix carried through the reaction sequence.

Determination of the enantiomeric purity of alcohol **2.22** was then carried out by its subsequent reaction with firstly (*S*)-, and then (*R*)-methoxy-α-trifluoromethylphenyl acetyl chlorides (MTPA-Cl) (Scheme 2.14),[38] to give **2.24** and **2.25** respectively.



Scheme 2.14. *Reagents and Conditions*: i. DMAP, Et_3N, (*S*)-MTPA-Cl, CH_2Cl_2, r.t., 5 min., 100%; ii. DMAP, Et_3N, (*R*)-MTPA-Cl, CH_2Cl_2, r.t., 5 min., 100%.

(*S*,*S*)-Mosher ester **2.24** was prepared by treatment of a solution of **2.22**, DMAP and triethylamine, in dichloromethane, with a solution of (*S*)-MTPA-Cl in dichloromethane. The reaction was then repeated using (*R*)-MTPA-Cl, to give the corresponding (*R*,*S*) isomer **2.25**, the ^{1}H NMR spectrum of which differed from that of **2.24**. In order to avoid kinetic resolution affecting the calculation of ee, the reactions were monitored by TLC to ensure complete consumption of the starting material. Note that the major isomer formed in the preparation of **2.24**, and any minor isomer formed in the preparation of **2.25**, are enantiomers, and therefore identical by ^{1}H NMR. The presence of any minor isomer can,

therefore, be observed by direct comparison of the ^{1}H NMR spectra of the respective crude reaction mixtures. Figure 2.19 shows representative spectra for the crude reaction mixtures of Mosher esters **2.24**, and **2.25**, derived from reaction of alcohol **2.22** with (*S*)-MTPA-Cl and (*R*)-MTPA-Cl respectively.

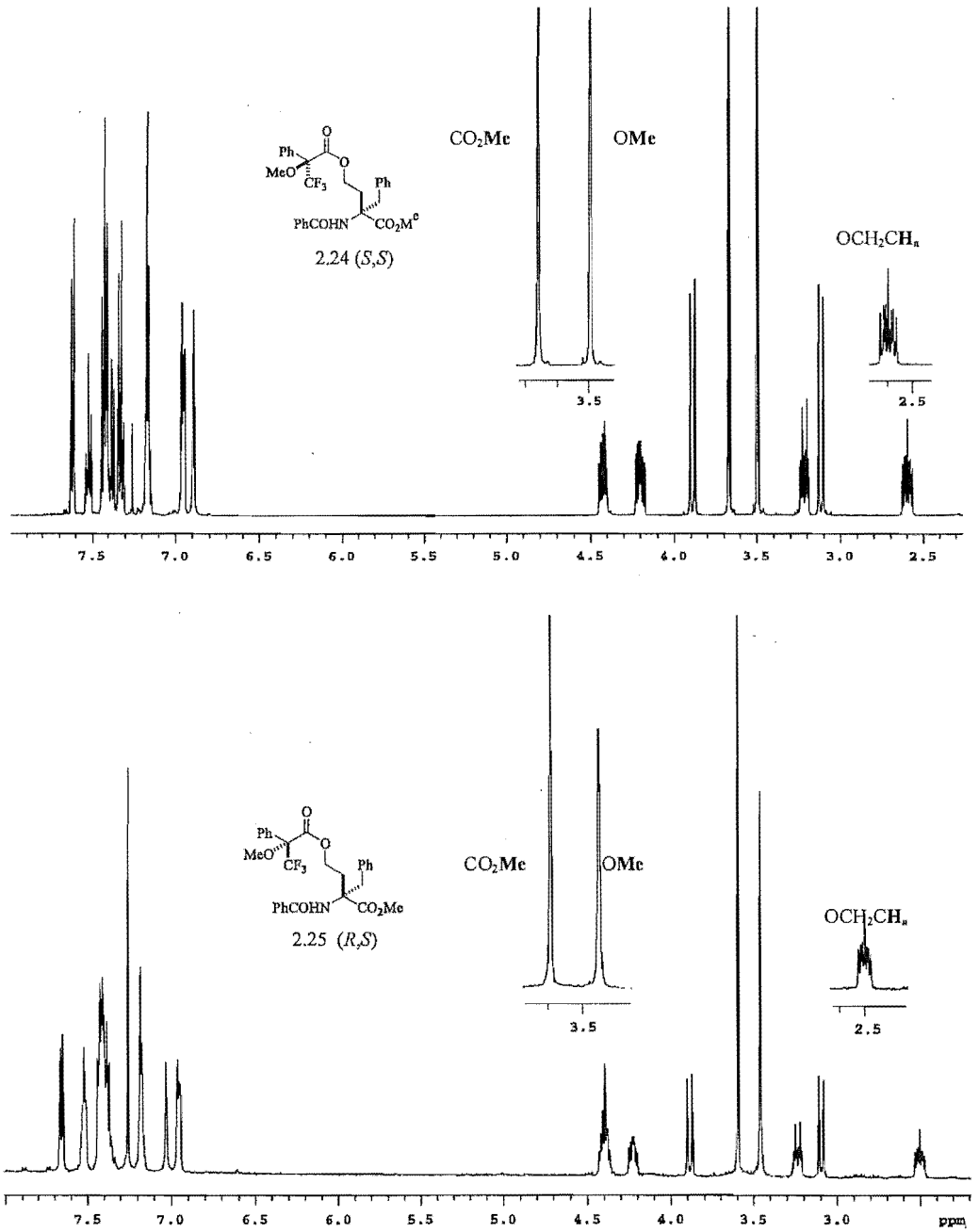


Figure 2.19. 500 MHz ^{1}H NMR spectra of the crude reaction mixtures of Mosher esters **2.24** and **2.25** respectively.

Key ^{1}H NMR resonances used in the comparison of Mosher esters **2.24** and **2.25** included those for OCH_2CH_a, COMe, and CO_2Me. Chemical shifts associated with these resonances correspond to δ 2.60ppm, 3.50ppm and 3.67ppm respectively for **2.24**, and δ 2.50ppm, 3.46ppm and 3.60ppm respectively for **2.25**. The minor isomers were not observed in either of the two Mosher ester reaction mixtures upon analysis of the respective ^{1}H NMR spectra (refer Figure 2.19). This equated to a >95% enantiomer excess for alcohol **2.22.** This is a key finding since it also establishes the enantiomeric purity of the key precursors **2.7**, and **2.5**. These compounds have also found use elsewhere in this thesis, in the enantioselective preparation of a potent thrombin inhibitor described in Chapter 4, and also a key cyclic amide described in Chapter 7.

2.7 Conclusion and Future Work

An enantioselective synthesis of a 1,2,3,6-tetrahydropyridine mimic of type **2.2** has been described. This sequence utilised a combination of Grubbs' RCM chemistry to give the cycle, and Seebach oxazolidinone chemistry to establish the absolute stereochemistry. Initial efforts focussed on the incorporation of a benzyl substituent at the α-carbon, leading to the preparation of the *L*-phenylalanine-derived tetrahydropyridine mimic **2.9**. The solid-state conformation of this new mimetic was also determined by X-ray crystallography, with it displaying significant conformational restriction about the amide bond. The torsion angle about the amide bond was found to be 38.7 (2) / 40.2 (2)° indicating that this mimic has potential for use as both a *cis*-amide bond mimic and a β-turn mimic.

Subsequent derivatisation of **2.9** was also undertaken. Hydrolysis of the methyl ester was carried out to give **2.15**, a compound suitable for incorporation into peptides via the C-terminus. Hydrogenation of the olefin of **2.9** gave the saturated piperidine derivative **2.12**. The solid-state conformation of **2.12** was obtained by X-ray crystallography revealing that, like **2.9**, significant conformational restriction existed about the amide bond. The amide torsion angle for **2.12** was found to be –47.7 (3)°, indicating that an inversion, or 'conformational flip', had taken place about the amide bond, with respect to **2.9**, as a result

of reducing the olefin. This suggested that peptides incorporating **2.9** and **2.12** would adopt significantly different conformations due to restraints imposed by the monomer's differing amide torsion angles.

Dihydroxylation and dibromination were also carried out on **2.9** to give the *cis*-dihydroxy- and *trans*-dibromo derivatives **2.13** and **2.14** respectively. Assignment of stereochemistry to the major isomers in these reactions was based largely on steric grounds, with approach of reagents predominantly occurring from the olefinic face opposite the benzyl group. Attempts to produce crystals suitable for x-ray crystallography proved initially unsuccessful, however in the case of **2.14a** a crystal was obtained after a period of 6 months. The solid-state conformation was obtained revealing that **2.14a** had undergone lactonisation to give the bicyclic lactone **2.16**. This transformation was thought to have occurred via hydrolysis of the methyl ester and subsequent nucleophilic attack of the corresponding acid group at C5. The absolute stereochemistry of **2.16** confirmed the assignment of the absolute stereochemistry of the major dibromide isomer **2.14a**, from which it was derived, as (4*S*,5*S*), an assignment that had previously been based on steric grounds.

Finally, the enantiomeric purity of the key intermediate **2.7**, and hence subsequent derivatives, was established. This was achieved by ozonolysis of **2.7**, followed by reduction, to give alcohol **2.22**, which was reacted with (*S*)- and (*R*)-MTPA-Cl respectively. An analysis of the product MTPA esters from these two reactions revealed a diastereomeric excess of >95%. This was an important result as it also established the enantiomeric purity of **2.5** and **2.9**, and confirmed the overall enantiomeric integrity of the synthesis.

Future work in this area could involve the incorporation of **2.9**, and its derivatives, into a range of peptides to explore their effect on secondary structure. Work centred on incorporating these procedures into solid phase protocols would provide ready access to peptides of this type. This sequence of reactions should also be amenable to the preparation of a range of tetrahydropyridine- and pyridine-based amino acid mimetics using

oxazolidinones derived from both natural and non-natural amino acids. Additional derivatisations of the olefin could lead to the establishment of a library of monomers, each with a unique and defined conformation, for use in the development of a range of potential peptidomimetics.

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CHAPTER THREE

SYNTHESIS OF α-SUBSTITUTED PYRROLINE AND PROLINE PEPTIDE MIMICS VIA RING–CLOSING METATHESIS

3.1 Introduction

A great deal of interest exists in the design and synthesis of conformationally restricted amino acid mimics for use in the development of peptidomimetics. Here we present the design, synthesis, and derivatisation, of a new class of α-substituted pyrroline-based peptide mimics, for use in this process.

Recently, attention has focused on the synthesis of simple cyclic monomeric units, for use in the construction of more complex peptidomimetics, biopolymers and peptide foldamers. The resulting compounds possess well defined, and stable, secondary structures and hence functions. An important natural example of this link between conformation and biological function, is evidenced by the ability of the pyrrolidine proline, and its derivatives, to induce secondary structure in peptides and proteins. While the majority of peptide sequences in proteins reside in helices or β-pleated sheets, the incorporation of proline, or its analogues, into polypeptides chains produces a striking effect on the conformation of its associated amide bonds. In a normal amide bond, the *trans*-conformation is energetically favoured over the *cis*-conformation by approximately 10 kcal/mol^{-1}, due to less steric congestion between adjacent amino acid side chains. However, where rotation about the N-Cα bond is restricted by the constituent cycle of proline, this is not always the case, with the amide bond preceeding proline in a linear peptide sequence often adopting a *cis*-conformation (Figure 3.1). As a result, Xaa[a]-*cis*-proline amides are seen in 10-30% of short propyl peptides, while a further 6% of Xaa-proline amide bonds in longer peptides are known to adopt the *cis*-conformation. This element of conformational restriction often results in a bend, or 'kink', in a linear peptide sequence.

[a] Where Xaa is any amino acid.

Figure 3.1. The *cis-trans* proline equilibrium.

As a consequence, proline plays an important role in the structure of compounds such as collagen, a structurally important, fibrous protein found in bone, teeth, blood vessels, connective tissue, tendons, cartilage and hide (Figure 3.2). Collagen consists of intertwined strands of proline-, and hydroxyproline-rich amino acid chains, that combine to form a unique triple helix. These triple helices then associate to form strong, inelastic fibrils, with various activities such as running and jumping made possible by their high tensile strength. Recently, it has been shown that modification of the peptide backbone of collagen can lead to a change in conformation and stability of these triple helices.[1] The preparation of proline derivatives has subsequently become an area of interest for the development of new and important biomaterials.

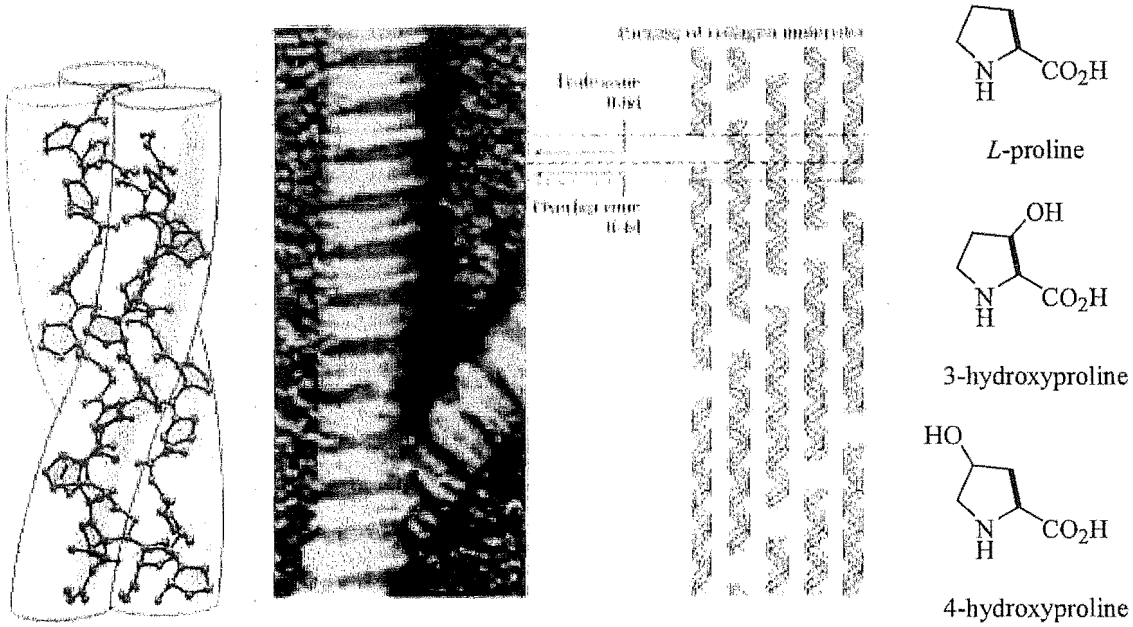


Figure 3.2. Collagen is an important example of the link between structure and function.

Hydroxyprolines,[2] can be found abundantly in a diverse range of other naturally occurring biologically active compounds.[3] *trans*-4-Hydroxy-*L*-proline, first discovered in gelatin hydrolysates in 1902, has since been found in numerous proteins. For example, it has been identified as a component of two structurally related lipopeptides from *Lyngbya majuscula*: majuscullamide D, which is cytotoxic,[4] and microcolin A, which has immunosuppressive, antileukemic, and protein kinase C inhibitory properties (Figure 3.3).[5,6]

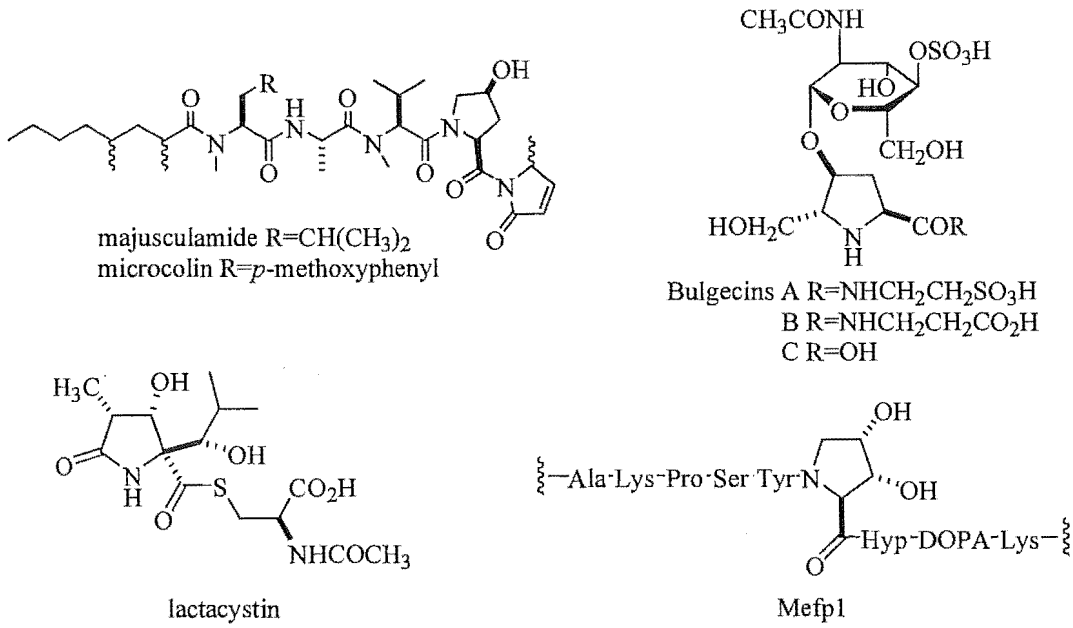


Figure 3.3. Examples of naturally occurring proline analogues

Other examples include bulgecins A, B and C, aminoglycoside antibiotics isolated from *Pseudomonas acidophila* and *Pseudomonas mesoacidophila*,[7] and lactacystin, a derivative of 3-hydroxypyroglutamic acid isolated from *Streptomyces* sp. OM-6519.[8-10] Three of the four possible isomers of 3,4-dihydroxy-*L*-proline have been identified in nature. The *L*-2,3-*cis*-3,4-*trans*-isomer was isolated from the cell wall hydrolysates of the diatom *Navicula pelliculosa* almost 30 years ago,[11-13] while the *L*-2,3-*trans*-3,4-trans isomer was isolated from the toxic mushroom *Amanita virosa* in 1980.[14,15] Finally, in 1994, the *L*-2,3-isomer was identified as the sixth residue in the repeating decapeptide sequence of Mefp1, an adhesive protein produced by the marine mussel *Mytilus edulis*.[16-18]

Other proline analogues of importance include 3,4-dehydro-*L*-proline **3.1** (Figure 3.5), a component of phomopsin A (Figure 3.4), the main mycotoxin isolated from the lupin *Phomopsis leptostromiforsis*.[19,20] The phomopsins inhibit polymerisation of tubulin and depolymerise preformed microtubules,[21] causing severe liver damage in cattle and sheep. Noformicin,[22] a potent antimicrobial and antiviral agent isolated from *Nocardia formica*,

contains a 5-iminoprolyl moiety, and exerts its biological activity through binding to the minor groove of DNA.[23]

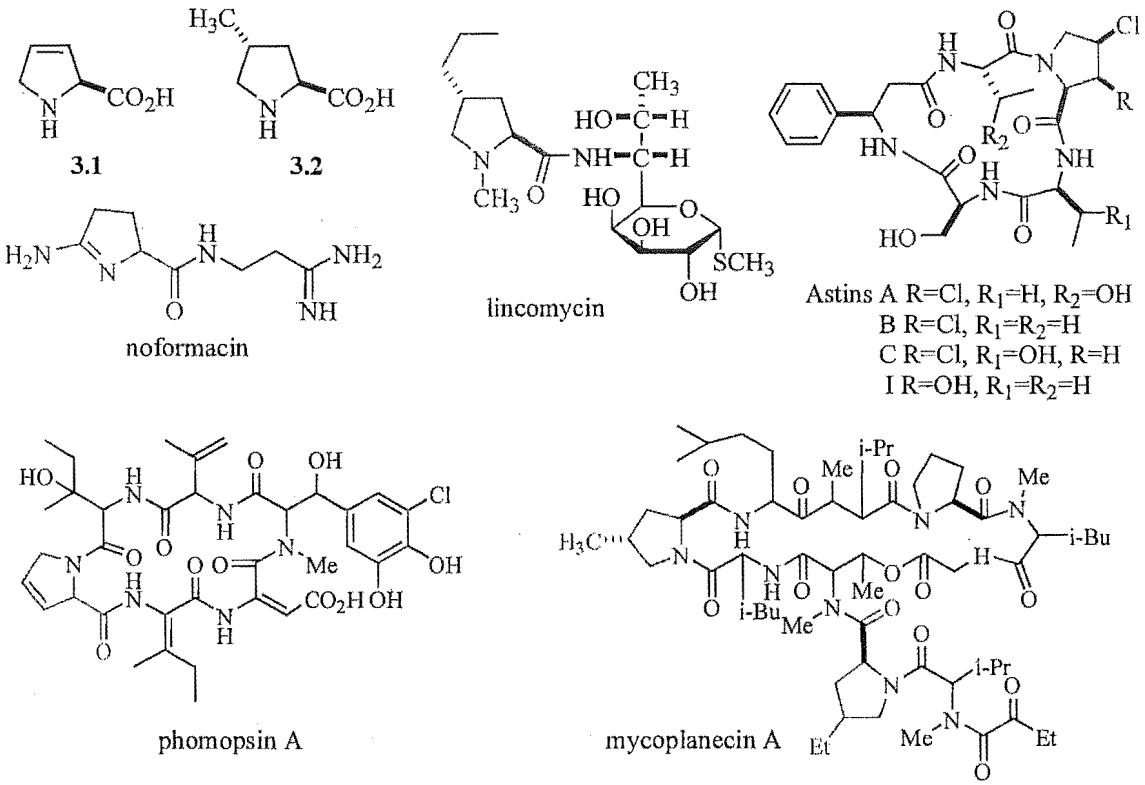
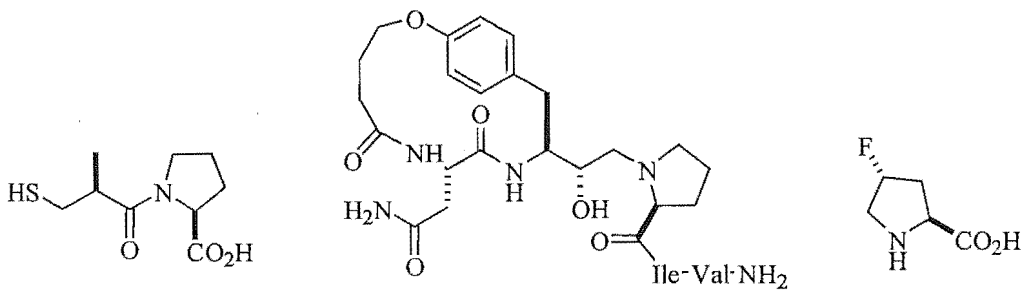


Figure 3.4. Further examples of naturally occurring proline analogues

Free *trans*-4-methyl-*L*-proline **3.2**, is an important component of a number of antibiotics, including mycoplanecin A, a depsipeptide isolated from *Actinoplanes awajinensis*, and found to be active against molds, yeasts and mycobacteria, including mycobacterium tuberculosis.[24] The clinically used broad spectrum antibiotic lincomycin, isolated from *Streptomyces lincolnensis*, *espinosus* and *variabilis*, consists of *N*-methyl-*trans*-4-*N*-prolyl-*L*-proline attached to an aminosugar.[25] Recently, astins A-C and I, cyclopeptides that contain chlorine substituted prolines, have been isolated from *Aster tataricus*,[26,27] and shown to possess potent antitumor activity.

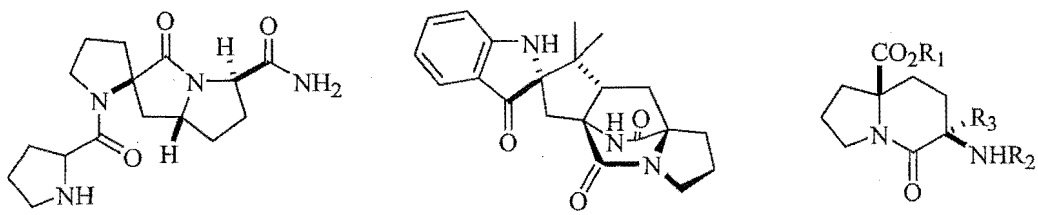
Proline analogues are also important components of a variety of synthetic peptidomimetics (Figure 3.5). Examples include captopril, a potent, and orally active antihypertensive agent,[28] and the potent HIV inhibitor **3.3**, [29] while the fluoroproline derivative **3.4** has been used in the study of collagen.[1]



Captopril **3.3** **3.4**

Figure 3.5. Examples of substituted prolines found in pharmacueticals

However, there are relatively few reported examples of proline analogues with a substitutent at the α-carbon.[30-32] Derivatives of the tripeptide Pro-Lys-Gly-NH_2 (PLG), a modulator of dopamine receptors in the central nervous system, has led to the development of compounds such as **3.5** (Figure 3.6), an extremely potent analogue, found to have 4 orders of magnitude greater activity than PLG itself.[33,34]



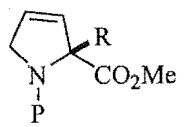
3.5 (-)-Brevianamide B **3.6**

Figure 3.6. Examples of α-substituted proline analogues

The brevianamides, paraherquamides, and asperparalines constitute an unusual family of fungal metabolites that possess a unique bicyclo[2,2,2]diazaoctane core ring system that has been proposed to arise in nature via a biological Diels-Alder reaction.[35] Bicyclic lactams of

type **3.6** have found use as novel XaaPro Type VI turn mimics, with various analogues evaluated as inhibitors of cyclophilin A, with K_d's ranging from as low as 5μM.[36] Significantly, syntheses of the α-substituted compounds shown in Figure 3.6, utilise chemistry pioneered by Seebach *et al.*, for the stereoselective alkylation of proline.[31,32] Synthesis of the α-substituted dehydro equivalents has also recently been reported.[30,37,38]

Given the prominent role of conformationally restricted amino acids and peptides in the design of peptidomimetics, and as part of a wider study towards the synthesis of a general class of α-substituted cyclic amino acids, we present here a new and versatile method for the stereoselective introduction of a substituent at the α-carbon of a proline analogue of type **3.7**.



3.7

As for the analogous piperidine series described in Chapter 2, the introduction of a substituent at the α-carbon would allow interactions usually associated with amino acid side-chains, i.e hydrogen bonding, π-π-stacking, hydrophobicity, hydrophilicity etc, to be incorporated into the mimic. Oxazolidinone chemistry developed by Seebach *et al.* was again utilised to introduce a substituent at the α-carbon, with ring-closing metathesis used in the formation of the heterocyclic ring. Inclusion of an olefinic moiety allows for subsequent functionalisation at this position, the result being a range of substituted proline analogues of interest in peptidomimetic design.

The key to the synthesis involves the introduction of a vinyl group by oxidative degradation of an alkylated methionine, followed by allylation on nitrogen, and finally RCM of the resulting diene as shown in Figure 3.7.

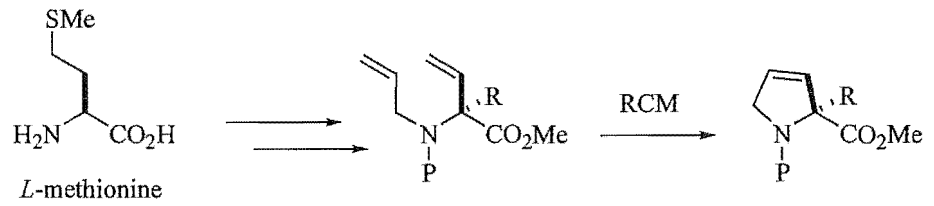
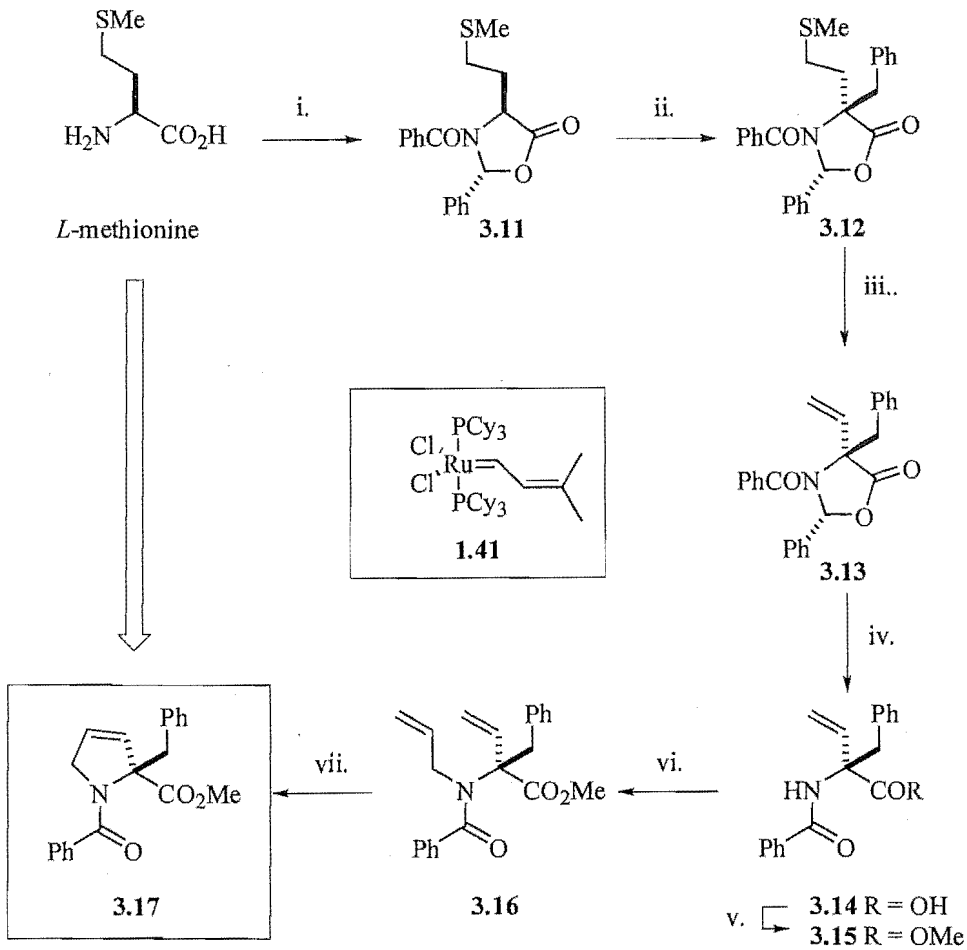


Figure 3.7.

As with the piperidine system (see Chapter 2), we envisaged that substitution at the α-carbon of the diene would further enhance ring closure in accordance with the Thorpe-Ingold effect. The oxidative degradation of methionine analogues has also been used in the preparation of aminocyclopentanecarboxylic acids described in Chapter 6.

We chose to introduce a benzyl group stereoselectively at the α-position of the proposed mimic, to give a phenylalanine analogue, that would allow direct conformational comparison with the previously prepared 6-membered series (see Chapter 2). The proposed synthesis of the pyrroline mimic is outlined in Scheme 3.1.

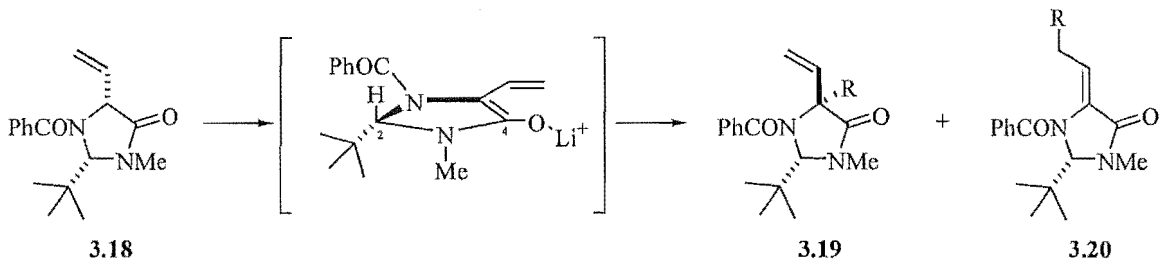


Scheme 3.1. *Reagents and Conditions*: i. a) NaOH, b) PhCHO, CH_2Cl_2, reflux, c) PhCOCl, CH_2Cl_2, 0° C for 4 h then rt for 16 h; ii.. base, THF, -78° C then benzyl bromide; iii. a) H_2O_2, AcOH, b) xylene, 200° C, sealed tube; v.. NaOH, MeOH, reflux; vi. CH_2N_2; vi. NaH, DMF, 0° C then allyl bromide; vii. **1.41**, benzene, reflux.

Here, *L*-methionine is protected as the diastereomerically pure *trans* oxazolidinone **3.11**, in a manner similar to the used during the piperidine synthesis detailed in Chapter 2 (Scheme 2.2). This would then be subjected to a stereoselective alkylation, with benzyl bromide, to give **3.12**. Oxidative degradation of **3.12** gives the vinyl oxazolidinone **3.13**, which would subsequently be hydrolysed, and alkylated at nitrogen, to give diene **3.16**. Ring closing metathesis would then give the desired pyrroline **3.17**. Note that the cyclic peptidomimetic **3.17**, possesses the same relative stereochemistry as the natural amino acid phenylalanine. As

with the piperidine series, this synthetic method should provide a convenient and general synthesis of pyrroline-based, and α-substituted proline-based, amino acid mimics from oxazolidinones derived from natural *L*-, and non-natural *D*-methionine.

In the proposed synthesis we were presented with either an alkylation-elimination, or an elimination-alkylation approach, in the preparation of **3.13** from **3.11**. Seebach *et al.* noted that alkylation of the vinyl imidazolidinones of type **3.18** (Figure 3.8) resulted in a mixture of diastereoisomers **3.19** being produced in moderate yield.[39]

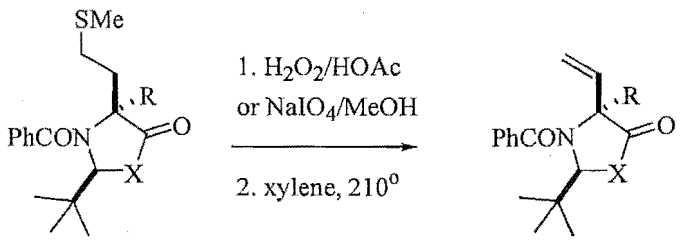


3.18 **3.19** **3.20**

Electrophile	Ratio **3.19**/**3.20**	Yield% **3.19**	%de
allyl bromide	>95:5	77	79
benzyl bromide	>95:5	75	87

Figure 3.8. Alkylation of vinyl oxazolidinones by Seebach *et al.*.

Additional studies with compounds of type **3.21** (Figure 3.9), revealed that, in contrast, the corresponding alkylated vinyl derivatives **3.22** could be obtained with little or no racemisation.[39]



3.21 **3.22**

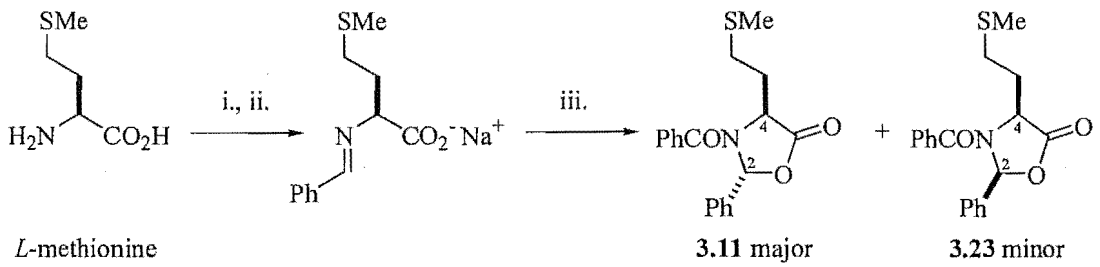
X	R	Yield%
NMe	Me	62
NMe	Et	75
O	H	13 (2S,4R) 26 (2S,4S)
O	Me	75

Figure 3.9. Oxidative elimination of methionine-derived α-substituted oxazolidinones.

It was therefore concluded, that the optimum order of transformations for the preparation of the alkylated vinyl oxazolidinone **3.13** from **3.11** (Scheme 3.1) would be via an alkylation-oxidative elimination sequence.

3.2 Synthesis of a Methionine-derived *trans* 5-Oxazolidinone

The synthesis began with the preparation, from *L*-methionine, of the *trans*-oxazolidinone **3.11** (Scheme 3.2).



Scheme 3.2. *Reagents and Conditions*: i. NaOH; ii. PhCHO, CH_2Cl_2, reflux; iii. PhCOCl, CH_2Cl_2, 0° C for 4 h then rt for 16 h.

Using the method of Seebach and Fadel,[40] the sodium salt of *L*-methionine was condensed azeotropically with benzaldehyde in dichloromethane to give the corresponding Schiff base. Acylation with benzoyl chloride, at 0°C for 4 h, followed by stirring at room temperature for 16 h, gave a crude mixture containing a 4:1 *trans* / *cis* diastereomeric mixture of phenyl-5-oxazolidinones **3.11**, and **3.23**. The diastereomers were subsequently separated by crystallisation from methanol to give the major isomer **3.11**, as a white solid, in 63% overall yield. Measurement of the optical rotation for **3.11** gave a $[\alpha]_D$ = +222°, c=1.0 $CHCl_3$ (lit. = +280.4°, c=1.0 $CHCl_3$). Crystals of the same sample gave a melting point of 157-159° C, which agreed with the literature value (157° C).[41]

The configuration of the major isomer **3.11** was assigned *trans* based on comparison of the ^{1}H and ^{13}C NMR data with that reported in the literature,[40,41] where the ^{1}H chemical shift of H2 (δ_H 6.71), for the *trans* isomer, lay significantly up field compared with that of the *cis* isomer. Further comparison with the *cis* and *trans* isomers of the 2-phenyl-oxazolidonones (see Chapter 2) and the related 2-(t-butyl)-oxazolidinones, revealed that this was invariably the case.[b,42]

The ^{1}H NMR spectrum of the *trans* oxazolidinone **3.11**, at 23°C, revealed broadening of the resonances for the H2 proton, the side-chain CH_2 protons, a number of aromatic protons, as well as the thiomethyl group. This is presumably due to restricted rotation about the amide bond. This was confirmed by determining the ^{1}H NMR spectrum at elevated temperature. Figure 3.10 shows the ^{1}H NMR spectrum collected for the *trans* oxazolidinone **3.11**, in $CDCl_3$, at both 23° C and 50° C (see also Chapter 2, Figure 2.7).

[b] Analysis, by the researchers, of alkylation products of both 2-phenyl-oxazolidinones, and 2-(t-butyl)-oxazolidinones, and comparison of the optical rotations of their derived amino acids, also confirmed the assignment of **3.11** as the major isomer.

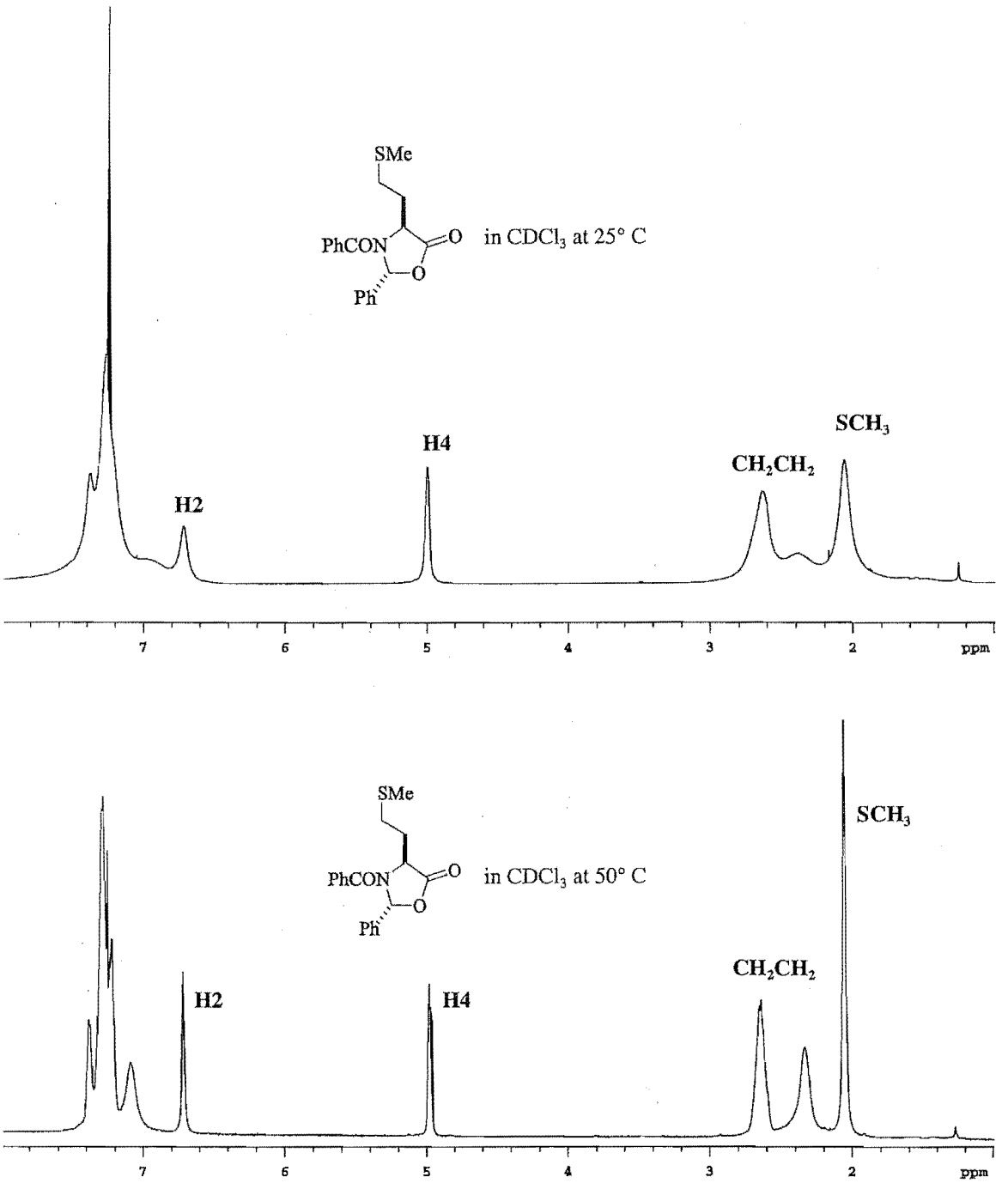
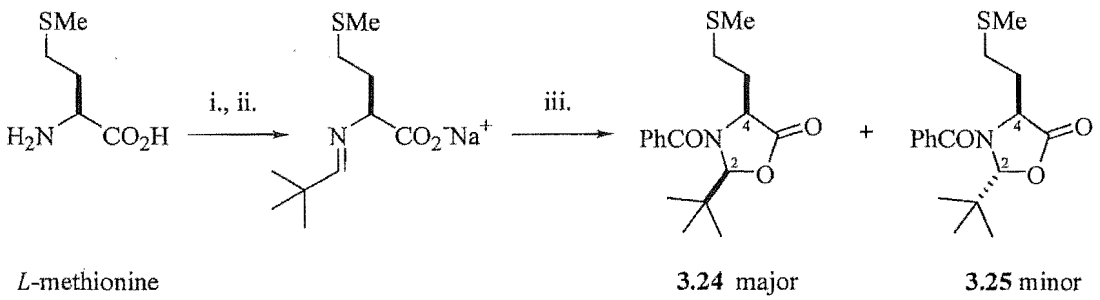


Figure 3.10. ^{1}H NMR spectra for the *trans* 5-oxazolidinone **3.11**, in $CDCl_3$, at 25° C and 50° C.

Increasing the temperature results in a noticeable sharpening of the resonances for H2, H4, the sidechain CH_2 protons and the thiomethyl protons.

The corresponding 2-(t-butyl)-oxazolidinone **3.24** was synthesised in a similar manner to that of **3.11** (Scheme 3.3), to illustrate that the *cis*-diastereoisomer was also accessible.



L-methionine **3.24** major **3.25** minor

Scheme 3.3. Reagents and Conditions: i. NaOH; ii. t-BuCHO, pentane, reflux; iii. PhCOCl, CH_2Cl_2, 0° C, 2 days.

Thus, the sodium salt of *L*-methionine was condensed azeotropically with trimethylacetaldehyde, in pentane, to give the corresponding Schiff base. Acylation with benzoyl chloride, at 0°C, followed by stirring at 0° C for 2 days, gave by ^{1}H NMR, a 5:1 *cis* / *trans* diastereomeric mixture of 2-(*tert*-butyl)-oxazolidinones **3.24**, and **3.25**. Crystallisation from methanol gave the major isomer **3.24** as a white solid in 71% yield, with the ^{1}H and ^{13}C NMR data corresponding to that reported in the literature.[40] Measurement of the optical rotation of **3.24** gave an $[\alpha]_D$ = +58° (c=1.0, $CHCl_3$), a value in close agreement with that reported in the literature (lit. = +62.2°, c=1.0, $CHCl_3$). The solid-state conformation of **3.24** was subsequently determined to allow confirmation of the relative stereochemistry. The absolute stereochemistry at C4 of **3.24** was assigned as *S* based upon the stereochemistry of the starting amino acid (*S*)-methionine. The crystal was found to possess a $P2_1$ space group, with 2 super-imposable molecules in the unit cell, indicating the **3.24** to be chiral. A perspective drawing of **3.24**, with atom labelling, is shown in Figure 3.11.

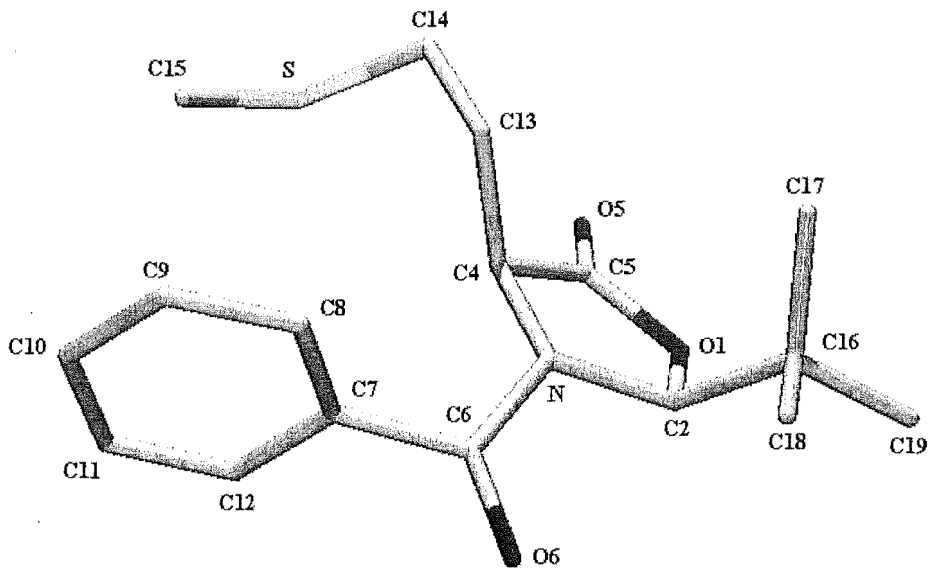
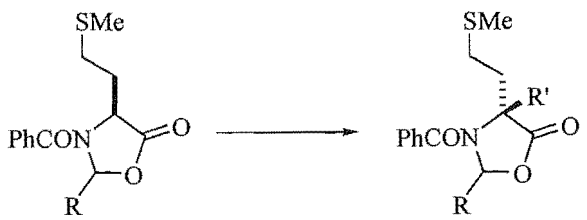


Figure 3.11. Solid-state conformation of the *cis*-2-(t-butyl)-oxazolidinone **3.24**.

The preparation of *trans*-**3.11** and *cis*-**3.24** represents an important result. Subsequent alkylation of these compounds allows access to either stereochemistry at C4, of the resultant α,α-dialkylated products. The high optical rotations of **3.11**, and **3.24**, together with the $P2_1$ space group obtained for the solid-state crystal structure of **3.24**, indicate that optically active samples of these compounds had been prepared for this purpose.

3.3 Synthesis and Solid-State Conformation of a Key Alkylated 5-Oxazolidinone

The introduction of the benzyl substituent at C4 of **3.11**, was next attempted. Seebach *et al.* noted that deprotonation of methionine-derived 2-phenyl-oxazolidinones and 2-(t-butyl)-oxazolidinones, at C4, requires the use of sterically non-hindered bases such as lithium diisopropylamide (LDA) and lithium diethylamide (LDEA) (Figure 3.12).[40,41]



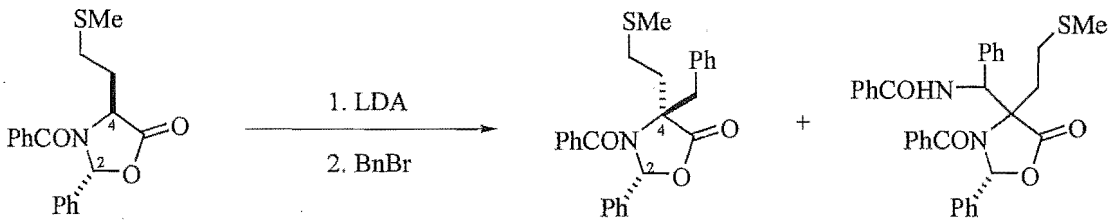
R	Base	R'	Yield%	ds
^{t}Bu (**3.24**)	LDEA	Me	69	-
Ph (**3.11**)	LDA	$BrCH_2CO_2Et$	85-94	>95%

Figure 3.12. Alkylation of methionine-derived 5-oxazolidinones

Based on these results, we first used LDEA in an attempt to effect alkylation of **3.11**. Thus, to a solution of diethylamine in THF, cooled to -78° C under argon, was added n-BuLi, and the solution was stirred at -78° C for 5min. A solution of **3.11**, in THF, was then added and the mixture was stirred at -78° C, for 40min, during which time the solution turned bright yellow. Benzyl bromide was then added and the reaction was stirred at -78° C for 2h and allowed to warm to rt overnight. However, upon workup, ^{1}H NMR analysis revealed that alkylation had not occurred, with **3.11** being recovered in quantitative yield. Repeated attempts rendered the same result.

LDA was then used in an attempt to effect alkylation of **3.11** (Scheme 3.4). Thus a solution of **3.11**, in THF, was cooled to -78° C under argon. LDA was added, and the resulting dark red/orange solution was stirred at -78° C for 15 min. Benzyl bromide was added and the reaction was stirred at -78° C for 2 h, then allowed to warm to rt overnight. Upon workup, ^{1}H NMR analysis of the crude mixture revealed the presence of additional aromatic resonances, a new pair of diastereotopic methylene resonances at δ_H =3.37 and 3.88ppm, and the absence of the H4 resonance at δ_H=5.00ppm associated with **3.11**. Subsequent purification, by silica chromatography, gave the dialkylated oxazolidinone **3.12** as a white solid in modest yield

(52%). Further elution also afforded the self addition product **3.30** (74mg, 2%) (refer Chapter 2.5 Self-Addition Products During the Alkylation of 5-Oxazolidinones)



(+)-**3.11** (+/-)-**3.12** **3.30** 2%

Scheme 3.4. *Reagents and Conditions*: LDA, THF, -78° C, 15min., then BnBr, -78° C to rt, 52% rac.

Measurement of the optical rotation of a recrystallised sample of **3.12** gave an $[\alpha]_D$ = 0.3°, C=1.0 $CHCl_3$.[c] The solid state crystal structure of **3.12** was determined, and satisfactorily refined, to further explore this lack of optical rotation, and establish the relative configuration. A perspective drawing of **3.12**, with atom labelling, is shown in Figure 3.13.

[c] For **3.11** $[\alpha]_d$ = +222°, c=1.0, MeOH.

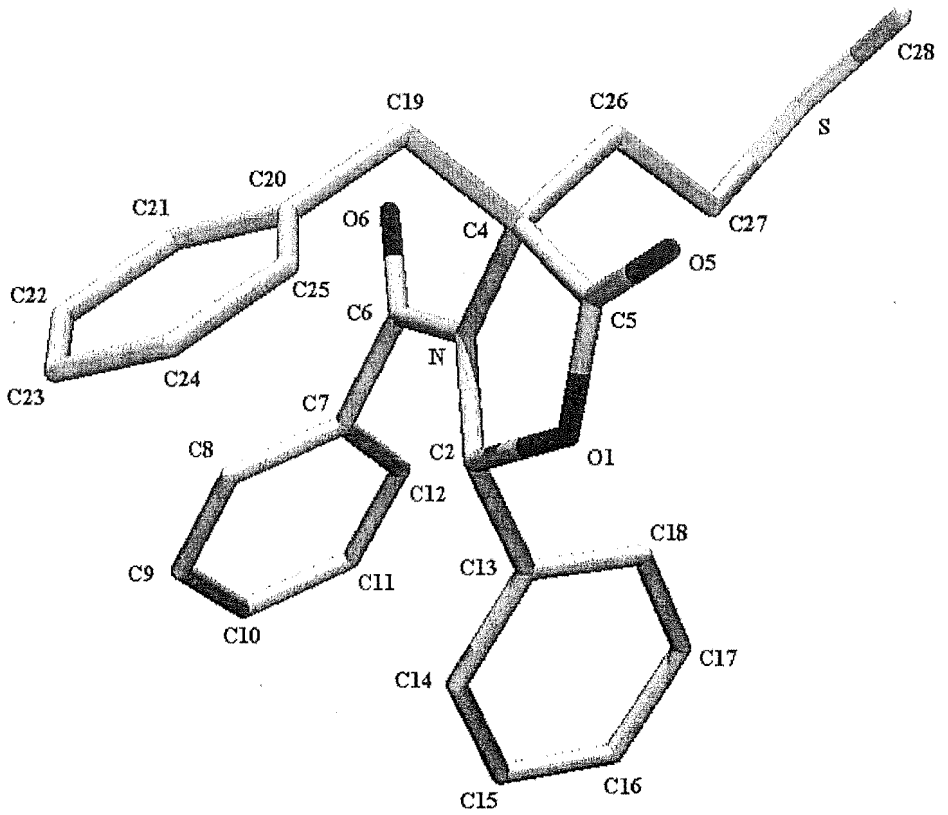


Figure 3.13. Solid-state structure of **3.12**, with atom labelling.

Analysis of **3.12** revealed one molecule in the asymmetric unit, with the oxazolidinone ring defined by O1-C2-N-C4-C5 adopting an essentially planar arrangement in the solid state. No significant pyramidisation of nitrogen was observed, with bond angles at N summing to 358.53°. It was observed that the C2 phenyl group, and the C4 benzyl group, existed in a *trans* relationship across the ring, revealing that alkylation had occurred at the face opposite the C2 phenyl group. Significantly, **3.12** was crystallised in the space group $P2_1/c$, indicating the compound to be racemic. This supported our previous observation arising from the sample's lack of optical rotation, and indicated that racemisation of the molecule had taken place. This suggests that scrambling at C2 must have occurred, followed by alkylation at the face opposite the C2 substituent, to give racemic **3.12**. The mechanism of this racemisation is

not yet understood, however as **3.11** has been shown to be optically active ($[\alpha]_D$ = +222°, c=1.0, $CHCl_3$), this scrambling is clearly subsequent to its formation, and the result of the reaction conditions used in the formation of **3.12**.

Having obtained **3.12**, and established the relative stereochemical outcome of the alkylation, we set about overcoming the problem of racemisation. Fortunately, a visit from Professor Koichi Narasaka, from the University of Tokyo, led us to try pyrrolidine as the amine used in the *in situ* generation of the lithium base.[d] Pyrrolidine represents a cyclic equivalent of the non-hindered base diethylamine, and has been used for the generation of lithium enolates in a variety of alkylation reactions (Figure 3.14).[43,44]

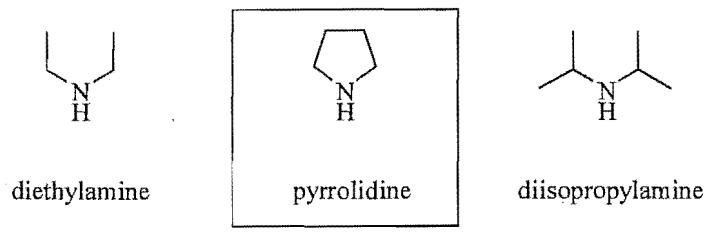
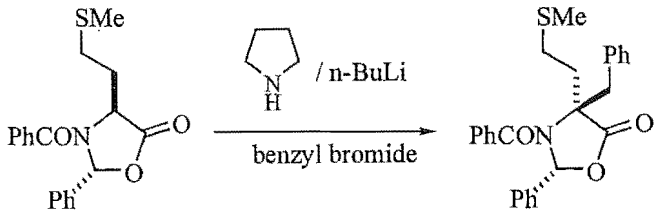


Figure 3.14. Pyrrolidine has been used in the generation of lithium enolates

Subsequently, alkylation of **3.11**, with benzyl bromide, in the presence of the lithium-pyrrolidine base was attempted (Scheme 3.5). *n*-Butyl lithium was added to a solution of pyrrolidine, cooled to -50° C in THF, and the solution was stirred for 30 min, following which **3.11**, in THF, was added. The reaction mixture was stirred for 20 minutes, whereupon benzyl bromide was added, and the solution stirred at -50° C for 1 h and then allowed to warm to room temperature overnight.

[d] Prof. Narasaka also suggested that in similar alkylations, where a non-hindered amine is required, pyrrolidine ought to be a first choice.

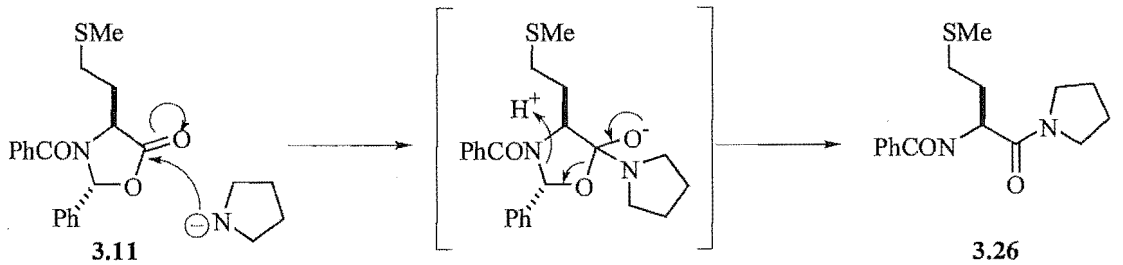


(+)-3.11 (+)-3.12

Scheme 3.5. Reagents and Conditions: pyrrolidine, n-BuLi, THF, -50°, 30 min., **3.11**, stir 20 min. at -50°C then add benzyl bromide, stir -50°C 1 h then at r.t. for 16 h, 61%.

Analysis of the ^{1}H NMR spectrum obtained for the crude mixture indicated the presence of the dialkylated product, with purification via silica-based chromatography giving **3.12** in 53% overall yield. The ^{1}H NMR spectrum was consistent with the absence of any minor diastereoisomer, with measurement of the optical rotation giving an $[\alpha]_D$=+14° (c=1.0, $CHCl_3$). It was concluded that alkylation, using the lithium-pyrrolidine base, had occurred stereoselectively, with the resulting configuration at C4 of **3.12** defined by the absolute configuration at C2 of the starting oxazolidinone **3.11**, which is in turn governed by the starting amino acid. The value of optical rotation obtained for **3.12**, is in line with that obtained for phenylalanine-derived analogue **2.5** described in Chapter 2 ($[\alpha]_D$ = -3°, c=1.0, $CHCl_3$), and suggested that alkylation had occurred with high stereocontrol. This was further supported when the solid-state crystal structure of the vinyl derivative **3.13** derived from it, was found to possess a space group indicating the molecule to be chiral (see Figure 3.15). Note also that the same sample of **3.11** used to prepare (+)-**3.12** in Scheme 3.5, was used in the reaction yielding (+/-)-**3.12** described in Scheme 3.4.

Preparation of (+)-**3.12** also yielded a by-product, subsequently assigned as compound **3.26**, which was isolated from the reaction mixture in 36% yield. The proposed mechanism of formation for **3.26** is shown in Scheme 3.6.

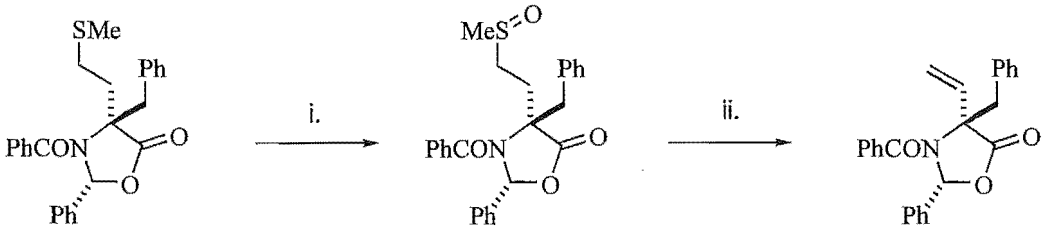


Scheme 3.6. Alkylation of **3.11** also resulted in the isolation of **3.26**.

The nucleophilic nature of pyrrolidine led us to conclude that nucleophilic attack had occurred at the carbonyl group of the oxazolidinone ring of **3.11**, resulting in ring-opening and loss of benzaldehyde. Suppression of this by-product was achieved by the addition of an excess of *n*-BuLi during the generation of the base, to yield after purification, **3.12** and **3.26**, in 61% and 23% respectively. Further rigorous optimisation of this reaction was not carried out as an ample supply of the dialkylated oxazolidinone (+)-**3.12** was obtained for use in subsequent steps.

3.4 Synthesis and Solid-State Conformation of a Key Vinyl-5-Oxazolidinone

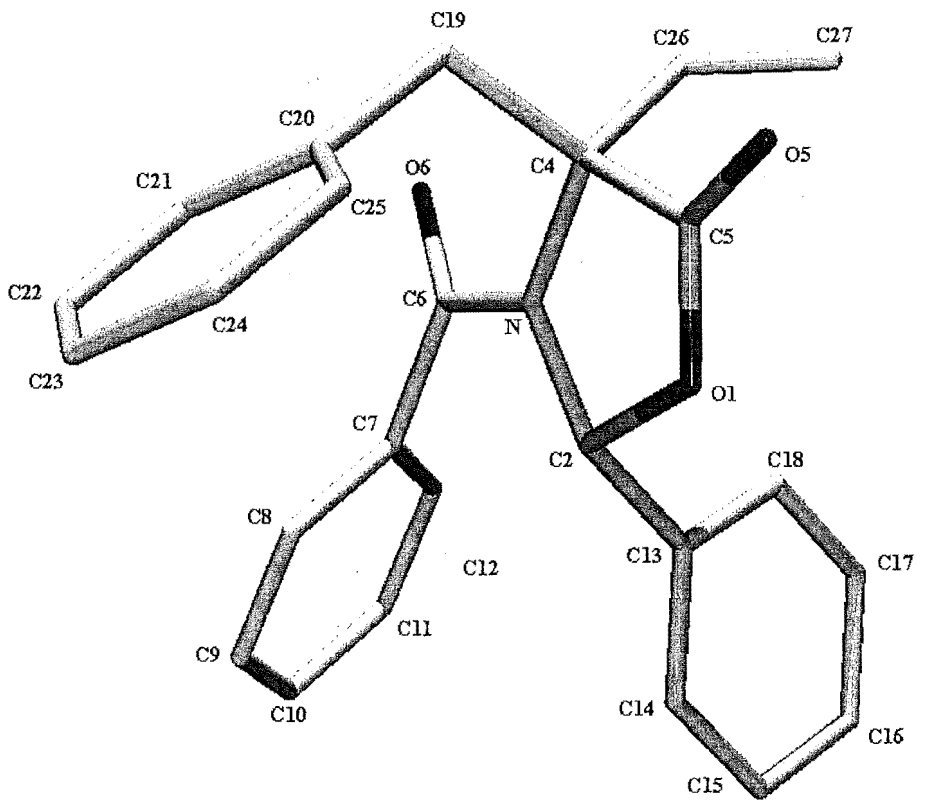
Oxidative degradation of the methionine side-chain of **3.12** was next carried out, using conditions described by Seebach *et al.* (Scheme 3.7). Hydrogen peroxide was added to a solution of **3.12**, in acetic acid, to form the sulfoxide **3.27**, which was subsequently dissolved in degassed xylene, and sealed in a glass tube under vacuum. The tube was then heated at 200° C for 16 h to give a dark brown solution, which upon purification via chromatography, gave the α,α-disubstituted vinyl oxazolidinone **3.13**, in 86% yield over two steps.



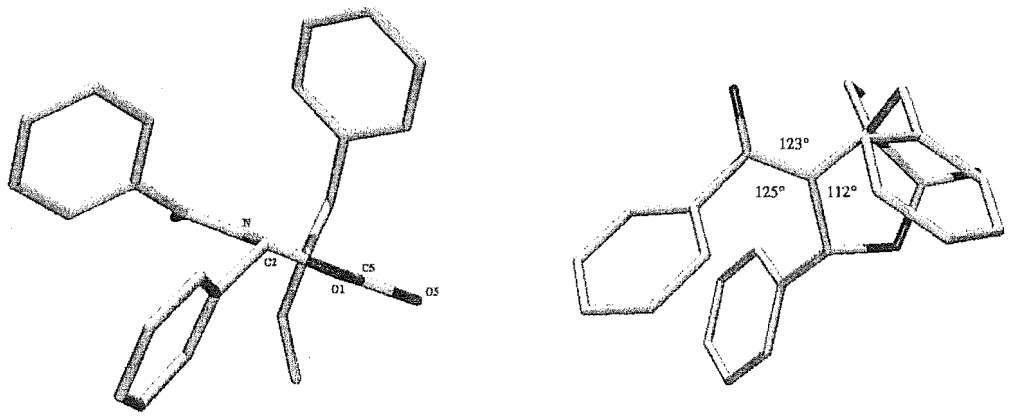
3.12 **3.27** **3.13**

Scheme 3.7. *Reagents and Conditions*: i. H_2O_2, AcOH, 94%; ii. xylene, 200° C, sealed tube, 93%.

Crystallisation gave **3.13**, as colourless crystals, from which the solid-state structure was determined and suitably refined. Perspective drawings of **3.13**, with atom labelling, are shown in Figure 3.15.



a)



b) c)

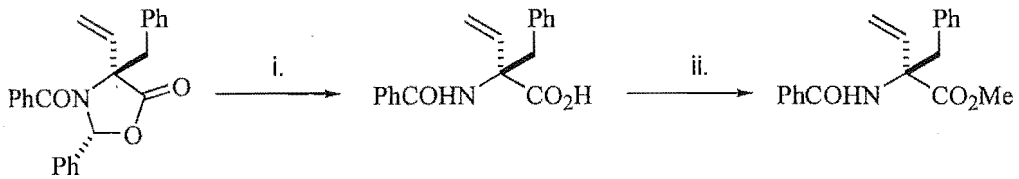
Figure 3.15. Perspective drawings of **3.13** with atom labelling: a) phenyl group at C2 and the introduced benzyl group at C4 adopt a *trans* configuration across the ring. b) the oxazolidinone ring depicted by O1-C2-N-C4-C5 adopts an essentially planar conformation. c) restriction occurs about C2-N-C4 with bond angles at N summing to 360.0°.

The phenyl group at C2 of **3.13**, and the introduced benzyl group at C4, existed in a *trans* relationship across the oxazolidinone ring, reconfirming the relative stereochemistry observed in the solid state structure of **3.12** (see Figure 3.13). The absolute stereochemistry of **3.13** was assigned based upon the structure of the *trans* oxazolidinone **3.11**, the absolute configuration of which is known, and defined by that of the starting amino acid (see Scheme 3.2). The oxazolidinone ring of **3.13** is shown to adopt an essentially planar conformation with the torsion angle defined by C4-N-C6-O6 having a magnitude of –6.9 (3)°, indicating a slight twist in the amide bond geometry. A distortion about nitrogen was observed, due to the oxazolidinone ring, with the bond angles defined by C2-N-C4, C4-N-C6 and C6-N-C2 having values of 112.07 (16)°, 122.9 (17)° and 125.03 (17)° respectively. No pyramidilisation was observed however, with the bond angles at nitrogen summing to 360.0°. Significantly, **3.13** crystallised in the space group $P2_12_12_1$, with 4 super-imposable molecules in the unit cell, indicating a single enantiomer had been obtained. This was supported by measurement of the optical rotation of **3.13**, which gave a value of $[\alpha]_D = +139°$, c=1.0 $CHCl_3$. Herein lies a key observation, with the chirality associated with **3.13**, coupled with its high optical rotation,

consistent with a high enantiomeric excess being obtained during its preparation, and also that of its precursor, **3.12**.

3.5 Synthesis and Solid-State Conformation of the α-Substituted Pyrroline Mimic

The vinyl-5-oxazolidinone **3.13**, was then hydrolysed with aqueous NaOH in refluxing methanol, followed by acidification, to give the free acid **3.14** (Scheme 3.8).



3.13 **3.14** **3.15**

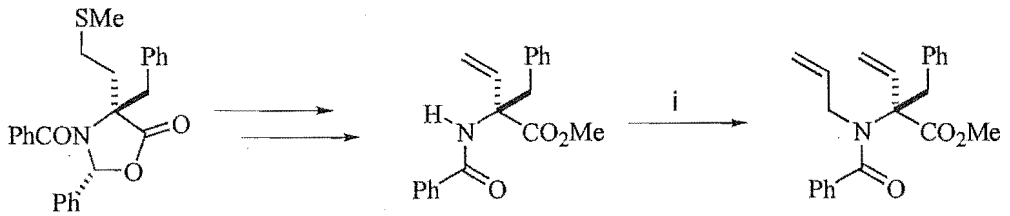
Scheme 3.8. *Reagents and Conditions*: i. NaOH, MeOH, reflux, 1 h; ii. CH_2N_2, 0° C to rt, 99% over 2 steps.

Subsequent esterification, with diazomethane, gave the α,α-disubstituted amino acid methyl ester **3.15**, in 99% yield over two steps. Measurement of the optical rotation for **3.15** gave a $[\alpha]_D = +54°$, (c=1.0, $CHCl_3$).

The preparation of the vinyl glycine derivative **3.15** represents a significant result. As in Chapter 2, the ability to introduce functional groups stereoselectively, in the formation of chiral building blocks, is of great importance in the synthesis of peptidomimetics. Here, **3.15** serves as the key precursor in the proposed synthesis of the chiral alkylated pyrrolidine and proline-based peptidomimetics of type **3.7** (refer introduction).

The synthesis of the proposed pyrroline mimic **3.17** (see Scheme 3.1) was then completed by first allylating the nitrogen of methyl ester **3.15** (Scheme 3.9). Deprotonation of **3.15**, with

sodium hydride in DMF, at 0°, followed by addition of allyl bromide, gave the dienic derivative **3.16**, which was isolated by silica chromatography in 31% yield.[e] Subsequent measurement of the optical rotation for **3.16** gave an $[\alpha]_D = -63°$, (c=1.0, $CHCl_3$).



3.12 **3.15** **3.16**

Scheme 3.9. *Reagents and Conditions*: i. NaH, allyl bromide, DMF, 0° C for 1.5 h. then rt for 30 min., **3.16** 31%, 51% recovered **3.15**.

3.5.1 RCM Studies

Diene **3.16** was then exposed to Grubbs' ruthenium alkylidine conditions,[45] at room temperature using Grubbs' ruthenium catalyst **1.40**, in an attempt to form the pyrroline mimic **3.17**. However, the starting diene **3.16** was recovered in quantitative yield. The reaction was repeated using a variety of solvents (CH_2Cl_2, toluene, benzene), and temperatures (rt, reflux), but again resulted in full recovery of the diene. Reaction with Grubbs' second generation ruthenium catalyst **1.42**, a compound reported to perform RCM at a rate of over a thousand times that of **1.40**, at both room temperature and at reflux, in CH_2Cl_2 and in benzene, was also unsuccessful, with the starting diene being recovered quantitatively (Figure 3.16).

[e] 51% of **3.15** was recovered during purification and this was cycled back through the reaction to obtain more diene.

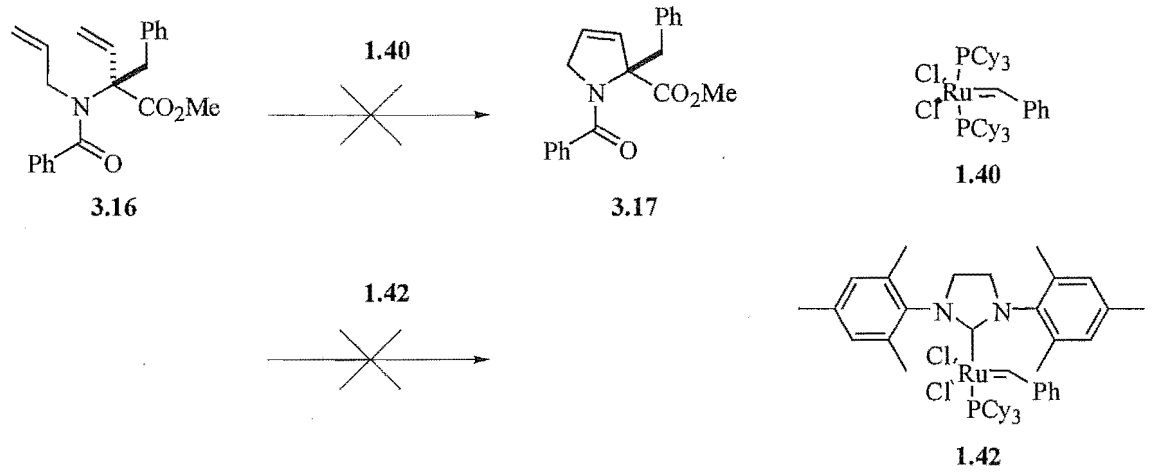


Figure 3.16. Attempted RCM of **3.16** using catalysts **1.40** and **1.42**, proved unsuccessful

In considering the factors affecting RCM, Grubbs *et al.* noted that the nature of the carbene ligand has a large influence on the initiation rates of RCM reactions, and even on the ability of catalysts to induce RCM at all. In reviewing this area Abell and Phillips noted that in many cases catalysts with extended carbene tethers (i.e. $CHCHCR_2$) effected RCM of more demanding substrates in the event that the more frequently used benzylidene catalysts could not.[46] This is consistent with catalysts of this type exhibiting a greater propensity for co-ordination of a hindered substrate olefin during formation of the intermediate metallocyclobutane. The inability of diene **3.16** to undergo RCM, while the analogous **2.8** (see Chapter 2) is high yielding, supports this concept (Figure 3.17).

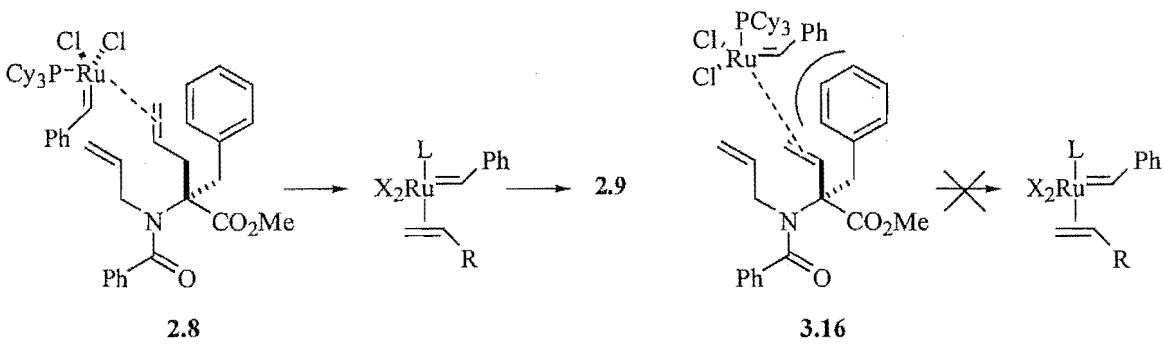
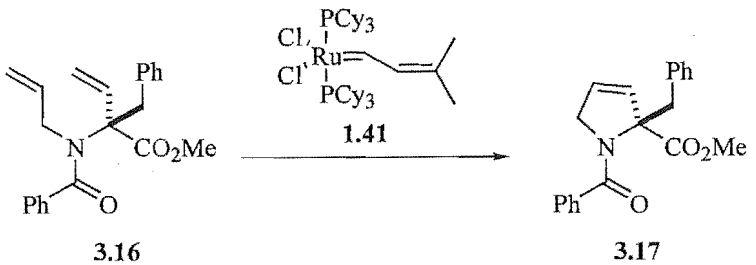


Figure 3.17. Interactions influencing RCM of **3.16**.

Subsequently, the methylbutenylidene ruthenium catalyst **1.41**, a carbene possessing an extended conjugated 'tether', was utilised in an attempt to effect RCM (Scheme 3.10).



3.16 **3.17**

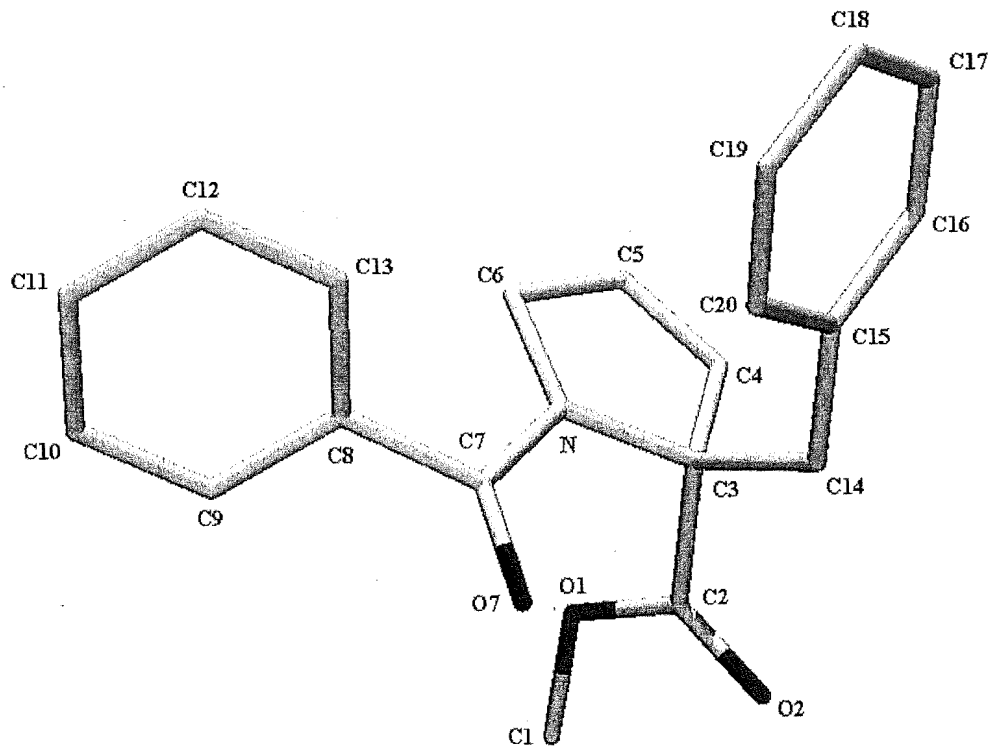
Scheme 3.10. *Reagents and Conditions*: 5 mol% **1.41**, benzene reflux, 16 h, 94%.

Thus, diene **3.16** was heated at reflux, in *dry degassed* benzene, in the presence of catalyst **1.41**. The reaction was followed by TLC and showed the gradual disappearance of the starting material, and subsequent formation of a new, more polar, compound. After 16h at reflux, isolation and purification via chromatography gave a white crystalline solid in 94% yield. ^{1}H NMR analysis of this solid showed the absence of the multiplets (δ_H= 4.99, 5.34, 5.36, 5.39, 6.22ppm), associated with the terminal olefins of **3.16**, and the presence of a singlet, integrating to two protons, at δ_H=5.71ppm. This indicated, to our satisfaction, that the desired ring closure had occurred to give the pyrroline mimic **3.17**. This result supported our earlier statements regarding the effect of extended carbene ligands on co-ordination, and subsequent RCM, of hindered olefins. Measurement of the optical rotation for **3.17** gave an $[\alpha]_D$ = -116.6° (c=1.0, $CHCl_3$). Note that the benzyl group of this cyclic peptidomimetic possesses the same relative stereochemistry as the natural amino acid phenylalanine.

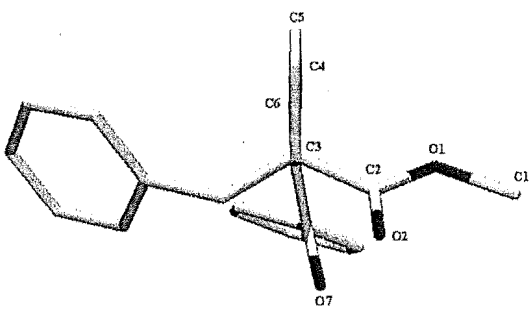
3.5.2 Solid-State Structure of the Pyrroline Mimic

Recrystallisation of a racemic sample of **3.17**, derived from racemic **3.12** (refer Scheme 3.4), from ethyl acetate / petroleum ether, allowed the solid-state crystal structure to be determined

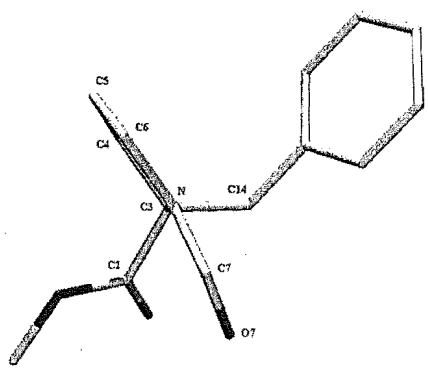
and satisfactorily refined. Perspective drawings of the solid-state structure of **3.17**, with atom labelling, are shown in Figure 3.18.



a)



b)



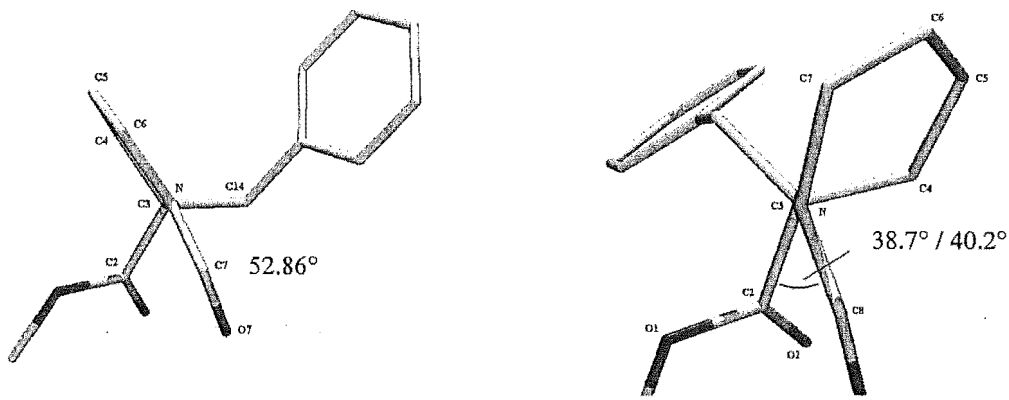
c)

Figure 3.18. a) Solid-state x-ray crystal structure of **3.17**. b) View showing the approximate planarity of the pyrrolidine ring. c) View along N-C3 axis indicating torsion angles about C8-N-C3-C2 peptide backbone are significantly shorter than that for proline.

An analysis of the solid state structure of **3.17** reveals that the proline-like N to Cα cyclisation results in significant restriction about the C7-N-C3-C2 peptide backbone in a manner similar to that observed for **2.9** (refer Figure 2.11). The torsion angle for **3.17** is 52.86 (13)°, a value significantly shorter than that for proline,[47,f] but longer than that of the tetrahydropyridine mimic **2.9**. The adjacent N-C3-C2-O1, C7-N-C3-C14 and C8-C7-N-C3 torsion angles are 44.99 (14)°, -68.67 (15)° and 176.79 (11)° respectively. The magnitude of the C8-C7-N-C3 and C7-N-C3-C14 torsion angles are consistent with a *Z*-amide bond with an *anti* relationship existing between the benzoyl and benzyl groups respectively. The pyrroline ring, represented by N, C3-C6, is essentially planar, with N deviating from the least squares plane defined by the other 4 atoms by 0.0365 Å. Some pyramidalisation of nitrogen was observed, with the bond angles at N summing to 358.55°, and C7 (the benzoyl group) deviating away from the plane of the pyrrolidine ring (see Figure 3.18c). Some twisting of the amide bond was also observed, with the magnitude of the torsion angle about C6-N-C7-C8 found to be –17.99 (18)°.

In summary, solid-state X-ray analysis reveals that compound **3.17** adopts a *cis*-amide bond geometry with significant conformational restriction about the amide bond. As with the tetrahydropyridine mimic **2.9** described in Chapter 2 (see Figure 2.11), **3.17** has potential for use as both a proline mimic, and β-turn mimic. Comparison of the solid-sate conformations of **3.17** and **2.9** reveals that, while the two mimics possess opposite stereochemistry at C3, and have opposite rotations (-116.6° and +38.2° respectively), both give a positive torsion angle value about the peptide backbone (see Figure 3.19).

[f] Proline angles vary depending on structure and environment ie they adopt a constrained but fluid system.



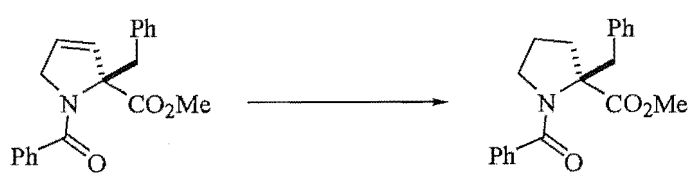
3.17 **2.9**

Figure 3.19. Both **3.17** and **2.9** maintain a positive torsion about the peptide backbone, despite possessing opposite stereochemistry at C3.

It is envisaged that the introduction of different substituents at the α-carbon of **3.17** would allow for 'fine tuning' of this torsion angle, and aid in maximising any receptor site interactions should these compounds be incorporated into peptides for biological study

3.6 Synthesis of the α-Substituted Proline Mimic

The olefin **3.17** was stirred, in deoxygenated methanol, in the presence of palladium-on-carbon, for 16 h under a hydrogen atmosphere. Filtration through Celite™ and purification via radial silica chromatography gave the α-substituted proline mimic **3.28** in 95% yield (Scheme 3.11), crystals of which were used to measure the optical rotation ($[\alpha]_D$ = -106.5°, $CHCl_3$).



3.17 **3.28**

Scheme 3.12. *Reagents and Conditions*: H_2, Pd-C, MeOH, 16 h, 95%.

3.7 Attempts to Establish the Enantiomeric Purity of a Key Intermediate

Synthesis of the pyrroline mimic **3.17** outlined in Scheme 3.1, and abbreviated in Figure 3.20, was successfully completed, with the stereochemistry and solid-state conformation being established by X-ray crystallography.

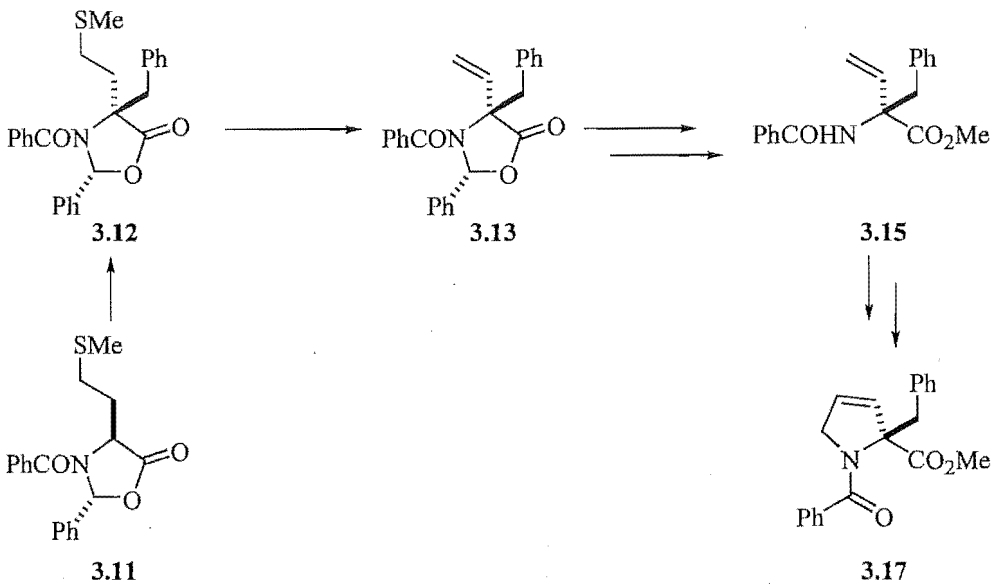
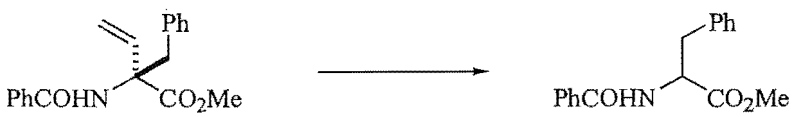


Figure 3.20. Synthesis of pyrroline mimic **3.17** from benzyl-5-oxazolidinone **3.11**.

The key to the enantioselective synthesis of **3.17** was the stereoselective alkylation of the 5-oxazolidinone **3.11**, in a manner similar to that described in the preparation of the piperidine analogue **2.9** in Chapter 2. Alkylation of **3.11** with benzyl bromide, gave the dialkylated 5-oxazolidinone **3.12**, the crystallisation of which, showed no sign of any minor diastereoisomer by ^{1}H NMR. Oxidative degradation of **3.12**, to give **3.13**, followed by hydrolysis and esterification, gave the key intermediate **3.15**, which was used in the synthesis of mimic **3.17**.

Repeated recrystallisation of **3.11** gave a sample that showed no sign of any minor diastereoisomer by ^{1}H NMR, and gave an optical rotation ($[\alpha]_D$ = +222°, c=1.0$CHCl_3$), slightly lower than that reported in the literature ($[\alpha]_D$ = +280.4°, c=1.0 $CHCl_3$).[41] However, this difference was consistent with that observed for **2.5** in chapter 2 (+267°, literature = +385.2°), a compound subsequently shown to be optically pure (see Chapter 2.6). To address this issue we set out to establish the enantiomeric purity of the key derivative **3.15**, in a manner similar to that described for **2.7** (see Scheme 2.12). Ozonolysis of **3.15**, followed by reduction of the resultant aldehyde, was envisaged to give an alcohol suitable for the preparation of Mosher esters (see Scheme 2.14 and Figure 2.19). However, attempts at preparing the aldehyde, via oxidation of **3.15** with ozone and solid $NaHCO_3$, at-78°C in THF, in a manner analogous to that described for **2.19** (Scheme 2.13), instead resulted in elimination of the vinyl group to give the *N*-benzoyl-phenylalanine methyl ester **3.29** (Scheme 3.11). Measurement of the optical rotation of **3.29** revealed a $[\alpha]_D$, in EtOH, essentially equal to zero, indicating the compound to be racemic.[g,48]



(+)-**3.15** **rac 3.29**

Scheme 3.11. Reagents and Conditions: O_3, $NaHCO_3$, CH_2Cl_2 / MeOH (3:1), -78° C then DMS.

Despite this, evidence for the enantioselective synthesis of **3.15** is amply supported by 1) the absence of any minor isomer being detected by ^{1}H NMR during the preparation of the 5-oxazolidinone **3.11**, and its associated high optical rotation ($[\alpha]_D$ = +222°, c=1.0 $CHCl_3$); 2) the absence of any minor diastereoisomer being detected by ^{1}H NMR during the preparation of **3.12**, and its associated optical rotation ($[\alpha]_D$ = +14.3°, c=1.0 $CHCl_3$); 3) the absence of any minor diastereoisomer being detected by ^{1}H NMR during the preparation of **3.13**, the

[g] *N*-Benzoyl-*L*-phenylalanine methyl ester, $[\alpha]_D$ = -45.3°, c=1.0 EtOH.

procurement of its solid-state structure, associated space group ($P2_12_12_1$), and optical rotation ($[\alpha]_D$ = +139.0°, c=1.0 $CHCl_3$); the optical rotations associated with subsequent derivatives of **3.13**, namely **3.15** itself ($[\alpha]_D$ = -54.1°, c=1.0 $CHCl_3$), **3.16** ($[\alpha]_D$ = -62.8°, c=1.0 $CHCl_3$), **3.17** ($[\alpha]_D$ = -116.6°, c=1.0 $CHCl_3$), and **3.28** ($[\alpha]_D$ = -106.5°, c=1.0, $CHCl_3$). These observations are consistent with those observed in Chapter 2, where the ee of analogous compounds was found to be >95% (Chapter 2.6).

3.8 Conclusion and Future Work

A synthesis of an optically active proline mimic of type **3.7** has been achieved. The sequence utilised a combination of Grubbs' RCM chemistry to form the cycle, and Seebach oxazolidinone chemistry to establish the stereochemistry. A benzyl group was successfully incorporated at the α-carbon, leading to the preparation of the pyrroline mimic **3.17**. The solid-state structure was determined for **3.17**, and showed a significant conformational restriction about the amide bond, with an amide torsion angle of +52.86 (13)°. This indicated that, as with the tetrahydropyridine mimic **2.9** described in Chapter 2, **3.17** has potential for use as both a *cis*-amide bond mimic and a β-turn mimic. Further comparison between **3.17** and **2.9**, revealed that, while the two mimics possess opposite stereochemistry at C3, and opposite optical rotations, both give a positive torsion angle value about the peptide backbone (Figure 3.19). Hydrogenation of **3.17** also yielded the α-substituted proline mimic **3.28**.

Determination of the enantiomeric purity of the key intermediate **3.15** was also attempted, however ozonolysis of **3.15**, in a manner similar to that used for the preparation of equivalent Mosher esters described in Chapter 2, instead resulted in formation of racemic *N*-benzoyl-phenylalanine methyl ester **3.29**. The solid-state structure of **3.13**, a key intermediate in the synthesis, its associated $P2_12_12_1$ space group, and the observations that all compounds in the sequence were optically active and contained no minor isomers by ^1H NMR, nevertheless

provided ample evidence to conclude that the synthesis of **3.17** had been stereoselective in nature. These observations were consistent with those in Chapter 2, where the ee of analogous compounds was found to be >95%

Future work could involve further derivatisation of **3.17** to obtain a library of monomeric compounds for incorporation into peptides, to examine their effect on secondary structure. The preparation of a range of mimics, derived from both natural and non-natural amino acids, could subsequently be used in the development of a range of potential peptidomimetics possessing defined conformation.

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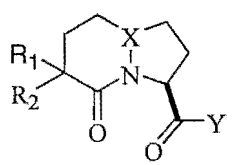
CHAPTER FOUR

ENANTIOSELECTIVE SYNTHESIS OF THE TETRAHYDROPYRIDAZINONE CORE OF A 2-OXO-1,6-DIAZOBICYCLO(4,3,0)-NONANE-9-CARBOXYLATE β-STRAND TEMPLATE

4.1 Introduction

Over 90% of all protein structure can be found in the form of common secondary structure motifs, or templates, such as reverse turns, β-strands and α-helices. These motifs provide the basis of a myriad of ligand-receptor, and enzyme-substrate interactions, for a multitude of biological processes. It has been known for some time that proteases bind their substrates, and inhibitors, by constraining them to adopt β-sheets / strands within the active site. This conformational requirement has been exploited in the design of inhibitors of the aspartic acid proteases renin,[1] and of HIV-1 protease. Cascading β-sheets have also been suggested as the cause of the insoluble amyloid fibrils associated with Alzheimer's disease.[2] In addition, protein-DNA interactions can occur with the protein interface adopting a β-strand conformation.[3] Due to the biological importance of these processes, much interest has been directed towards the development of mimics for these secondary structure motifs. A number of combinatorial libraries have been developed in this regard, to explore these biological structure-function relationships.

One such class of mimic are the 2-oxo-1,6-diazobicyclo(4,3,0)nonane-9-carboxylate dipeptidomimetic scaffolds of type **4.1**, that can be found in extended β-strand mimetics and other bioactives.[4]



4.1 X=N
4.2 X=CH

These scaffolds, together with those of type **4.2**, are designed to mimic the extended β-strand conformation adopted by substrates in the active site of a variety of serine proteases.[5] Homologation studies of these proteases have revealed a conserved trypsin-like core, with key insertions leading to a modification of substrate specificity. Inspection of numerous enzyme / substrate, and enzyme / inhibitor, X-ray crystal structures has highlighted the extended strand motif adopted by the inhibitor / substrate in the active site.[6] Inhibition of certain enzymes of this type is envisaged to be beneficial in treating their

associated disease states. Preliminary reports suggest that the selectivity displayed by compounds of type **4.1**, for one protease over another, is influenced by the nature of the substituents on the 2-oxo-1,6-diazobicyclo[4,3,0]nonane-9-carboxylate core.[7] The recent screening of a library of such compounds against a range of serine proteases supports this observation, with the identification of a number of potent and selective inhibitors.[8] One such target for these inhibitors has been the serine protease thrombin, a key enzyme in the blood coagulation cascade.

Thrombin is grouped among a class of enzymes referred to as 'protective' enzymes and plays a number of key roles in the blood coagulation cascade (See Figure 4.1).[9,10] Along with fibrinogen, thrombin is involved in preventing blood loss in the event of circulatory system damage, by forming blood clots at the site of injury. Specifically, it catalyses the conversion of fibrinogen to fibrin, one of the two major components of a blood clot, or thrombus. This conversion is brought about by the selective cleavage of key Arg-Gly peptide bonds, within fibrinogen, to form fibrin.[11]

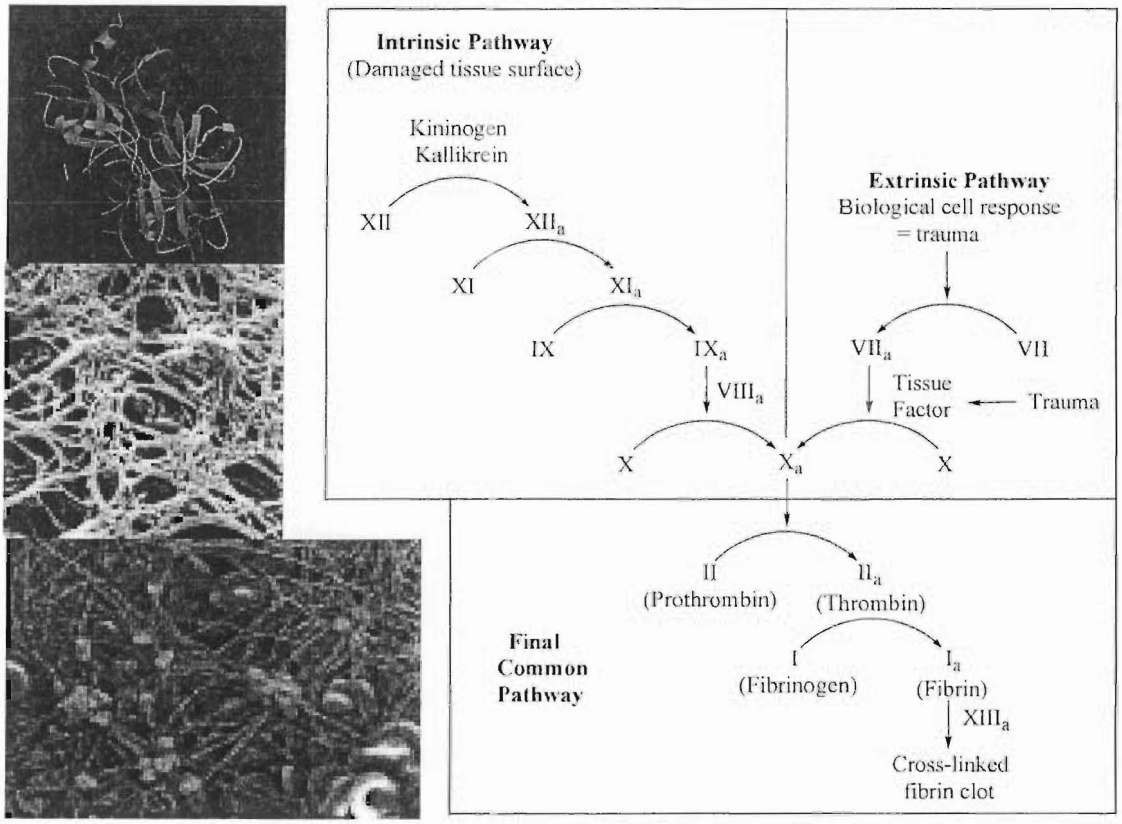
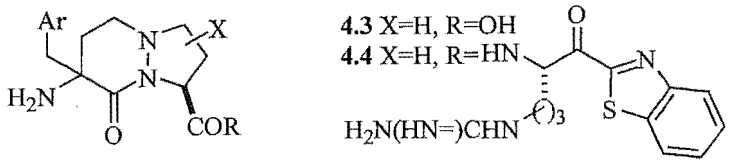


Figure 4.1 The blood coagulation cascade. The intrinsic and extrinsic pathways converge at Factor X, with the final pathway involving the activation of thrombin. This converts fibrinogen into fibrin, which aggregates into a cross-linked filamentous array to form the clot. Above-left. Ribbon diagram of thrombin. Left and below left. Electron micrographs displaying the filamentous nature of a blood clot.

Thrombin is also among one of the most potent known stimulators of platelet aggregation and thereby leads to the activation of the second major component of a blood clot. Thrombin directly activates Factor XIII to Factor XIIIa, resulting in covalent cross-linking of fibrin. Fibrin then readily aggregates into ordered fibrous arrays, stabilised by these cross-links, thereby helping to stabilise the growing clot. Finally, thrombin is self-regulatory. It catalyses its own synthesis via the activation of Factor V and VIII, and regulates its own activity via generation of the anticoagulant Activated Protein C.

Recently groups led by Boatman, Klan and Takahashi have used the diazobicyclic template **4.3** in the preparation of inhibitors for a range of serine proteases. In particular

compounds such as **4.4** were designed as, and subsequently found to be, potent inhibitors of thrombin.[5,7,8]



The template was designed as a mimic for the extended β-strand secondary structure adopted by fibrinogen in the active site of thrombin.[6,12] The bicyclic ring structure serves to rigidify the peptide backbone of the inhibitor, while placing the functional groups in approximately the same orientation as an idealised peptide (Figure 4.2).

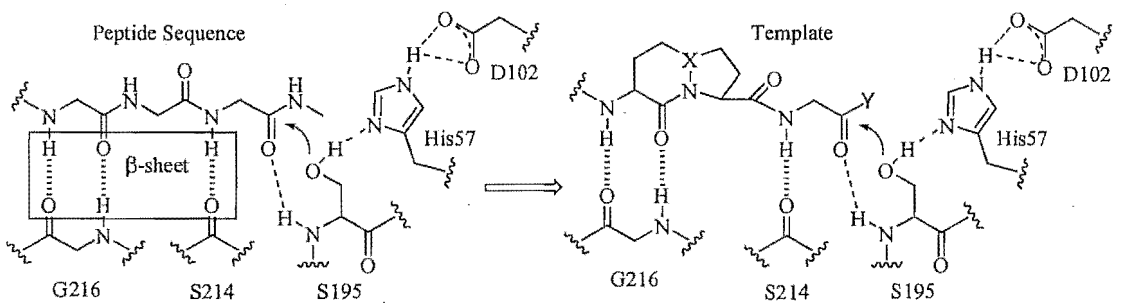
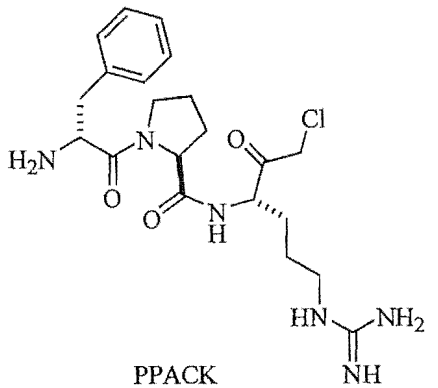


Figure 4.2. Template **4.3** was designed to mimic the β-strand structure adopted by fibrinogen, thrombin's natural substrate.

This strategy has been used extensively in the preparation of potential β-turn mimetics.[4] β-Turn mimetics of this type generally encompass the i+1, and i+2, residues of the turn, and position the i, and i+3, residues for formation of a hydrogen bond between the respective amide NH and carbonyl. However, bicyclic structures such as **4.1** and **4.2** also orient the atoms encompassed by the template in positions that are approximately the same as those in an extended strand. These properties have led to the recent use of compounds of this type in the preparation of β-strand mimetics. In the development of template **4.3**, the researchers chose substituents to match those of the D-Phe-Pro portion of PPACK, a known inhibitor of thrombin, and a compound that had earlier reached phase II clinical

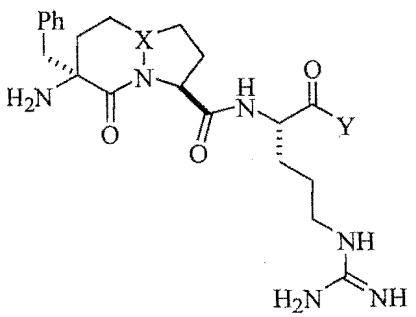
evaluation. This led to a number of compounds being identified that were not only potent, but very selective inhibitors of thrombin (i.e. **4.4**).[5,7,8]



PPACK

Table 4.1 illustrates extensive *in vitro* assay results for a number of these inhibitors against a range of coagulation and anticoagulation enzymes.

Table 4.1. Inhibition and Selectivity of Coagulation vs Anticoagulation enzymes.[5]



MOL098 X=N, Y=CH_2Cl

MOL144 X=N, Y=2-benzothiazolyl

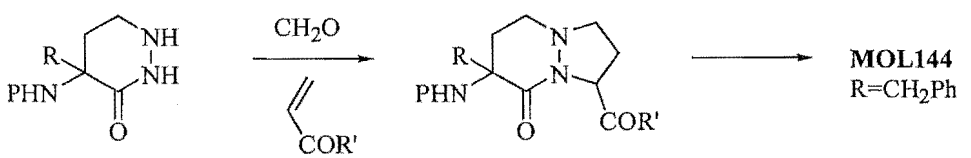
MOL174 X=CH, Y=2-benzothiazolyl

Enzyme[a]	PPACK[b]	MOL098[b]	MOL144[c]	MOL174[c]
thrombin	1.5	1.2	0.65	0.85
trypsin	ND	ND	0.64	0.23
Factor Xa	165	385	270	19.3
Factor VIIa	200	140	270	200
Protein C	281	528	3320	1250
plasmin	699	978	415	251
urokinase	508	927	600	325
t-PA	106	632	495	93

[a] Inhibitor concentration (selectivity=K_i other/K_i thrombin), [b] IC_{50} (nM), [c] K_i (nM)

Comparison of the data for PPACK with those of the constrained β-strand inhibitors indicated that the template imparted greater selectivity for thrombin than the more flexible peptide. MOL144 and MOL174 were further evaluated in an *in vivo* study revealing that both compounds were very effective in blocking platelet disposition. The bioavailability of these two compounds was also evaluated in rats and non-human primates, with MOL144 approximating 25% in rat and primate, while MOL174 approximated 2% in both species. This indicated that although MOL144 and MOL174 displayed similar characteristics in the *in vitro* assays, MOL144, which incorporated a diazobicyclic template, was consistently at least 10-fold more bioavailable *in vivo*.

Important precursors in the synthesis of the diazobicyclic template core of type **4.3**, and the associated inhibitors, are the tetrahydropyridazinones of type **4.5** (Scheme 4.1). To date substituted compounds of this type, where R is other than H, have only been synthesised as racemic mixtures, a point of note when considering the synthesis of subsequent templates. These mixtures are then subjected to a regioselective 1,3-dipolar cycloaddition to give the racemic bicyclic template **4.3**.[5]



4.5 **4.3**

Scheme 4.1. Templates of type **4.3** are formed via a 1, 3-dipolar cycloaddition reaction.

Final extension of the peptide chain yields a mixture of diastereomeric β-strand mimetics, such as MOL144, that must be separated by HPLC.[5] This basic methodology has also recently been extended onto solid phase to provide access to small libraries of β-strand mimetics as mixtures of isomers.[5,7,8]

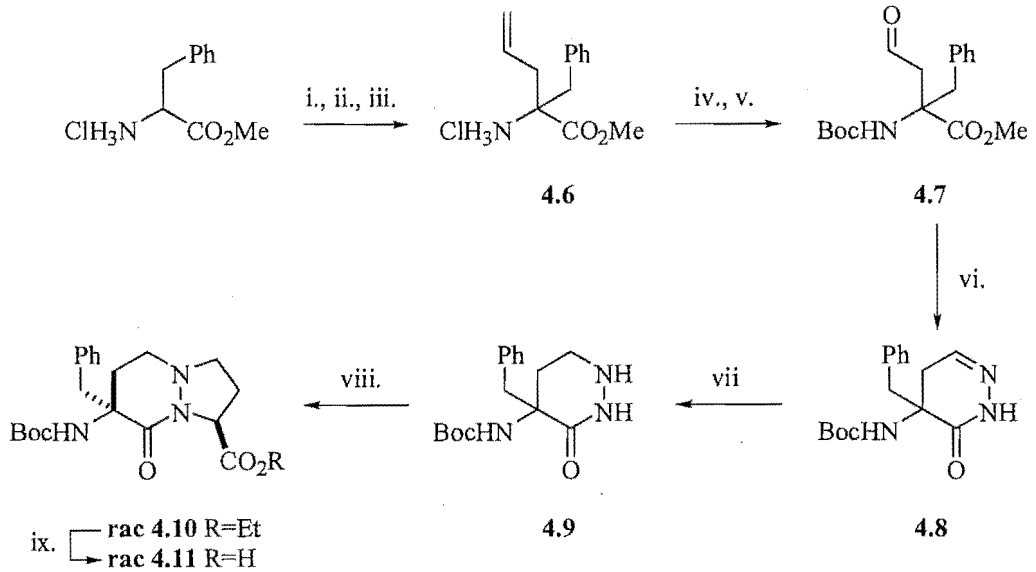
Here lies a deficiency in the synthesis. While the generation of isomers is sometimes desirable to explore conformational constraints, once the necessary stereochemical requirements for a template are determined, only compounds with the required

configuration can be used in the preparation of subsequent inhibitors. For thrombin, this corresponds to a *D*-configuration for the P_3 substituent being required to induce inhibitory activity. An opportunity therefore arose for the development of an asymmetric synthesis of compounds of this type, for use in peptidomimetic design.

Subsequently, efforts were made to develop an enantioselective synthesis of a peptidic tetrahydropyridazinone ring system, of type **4.5**, from (*S*)-phenylalanine (R=CH_2Ph). Derivatives of this type could then be utilised in the preparation of bicyclic peptidomimetics of type **4.3**, in what would be the first enantioselective synthesis of this important class of β-strand mimetic.

4.2 Synthesis of the Tetrahydropyridazinone Core

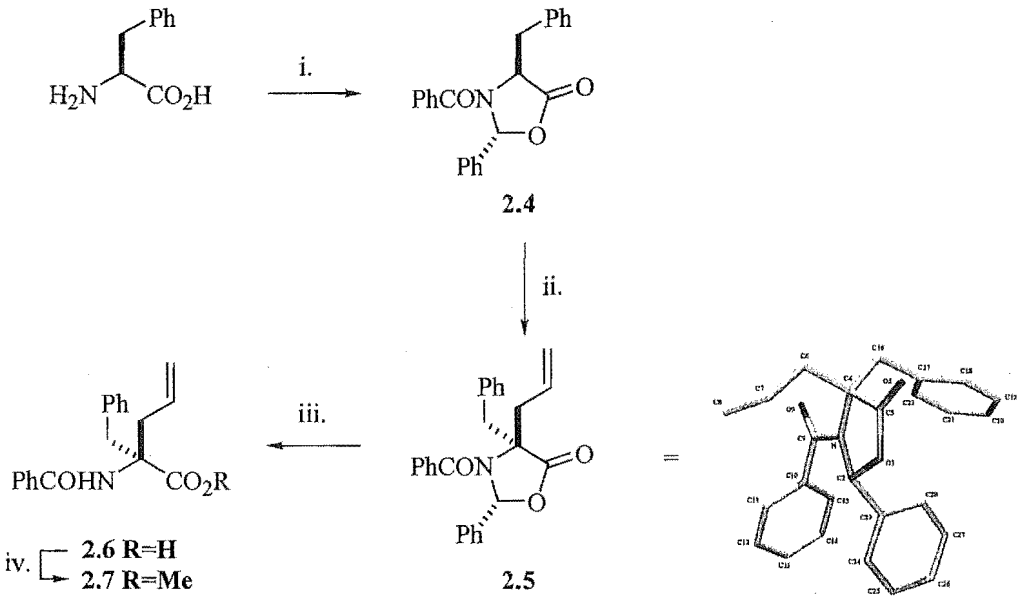
We began by considering the existing synthesis of the racemic phenylalanine-derived tetrahydropyridazinone template **4.10** (Scheme 4.2).[5]



Scheme 4.2. *Reagents and Conditions*: i. PhCHO, Et_3N, CH_2Cl_2, rt; ii. LDA, -78° C, then allyl bromide, -78° C to rt; iii. 1 M aq. HCl, MeOH, rt, 1 h; iv. Boc_2O, $NaHCO_3$, THF / H_2O, rt, 3 days; v. O_3, $NaHCO_3$, CH_2Cl_2 / MeOH; vi. Hydrazine, THF, reflux, 3 days; vii. Pt_2O (cat.), H_2, MeOH, rt, 48 h; viii. HCOH, ethyl acrylate, reflux; ix. LiOH, THF, rt, 1.5 h.

Here the benzaldimine of phenylalanine methyl ester was formed and alkylated with allyl bromide, with the imine then being hydrolysed to give (+/-)-α-allylphenylalanine methyl ester **4.6**. Subsequent protection as the *N*-Boc derivative, followed by ozonolysis of the olefin, gave aldehyde **4.7** which, when treated with hydrazine, gave the cyclic hydrazone **4.8**. Reduction of the cyclic hydrazone gave the tetrahydropyridazinone **4.9** as a racemate. This then underwent a 1,3-dipolar cycloaddition, when treated with formaldehyde in an excess of ethyl acrylate, to give a mixture of diastereomeric and regioisomeric products. The desired bicyclic template **4.10** was then isolated from this mixture, albeit in racemic form. Hydrolysis of **4.10**, to give **4.11**, allows for chain extension in the C-terminal direction and subsequent incorporation of the template into potential inhibitors.
From an asymmetric standpoint, the key feature of this synthesis is the non-stereoselective alkylation of phenylalanine to give the racemic allylphenylalanine derivative **4.6**. It was felt that if this key α,α-disubstituted amino acid could be obtained in an enantiomerically pure form, it could be taken through the sequence shown in Scheme 4.1 to give the bicyclic template **4.10** as a single isomer.

The key to our synthesis was the use of chiral oxazolidinone chemistry to generate enantiomerically pure α,α-disubstituted amino acids of type **4.6**. We had previously used this chemistry to develop an enantioselective synthesis of phenylalanine-derived tetrahydropiperidine mimetics of type **2.9**. (see Chapter 2).[13] There we had successfully alkylated (*S*)-phenylalanine, stereoselectively, to form compound **2.7**, an enantiomerically pure, *N*-benzoyl protected equivalent of compound **4.6** (refer Schemes 2.2 and 4.1). The enantiomeric synthesis of this key α, α-disubstituted amino acid is outlined in Scheme 4.3, with the absolute stereochemistry of **2.5** having been previously determined by X-ray crystallography (see Chapter 2).[13]

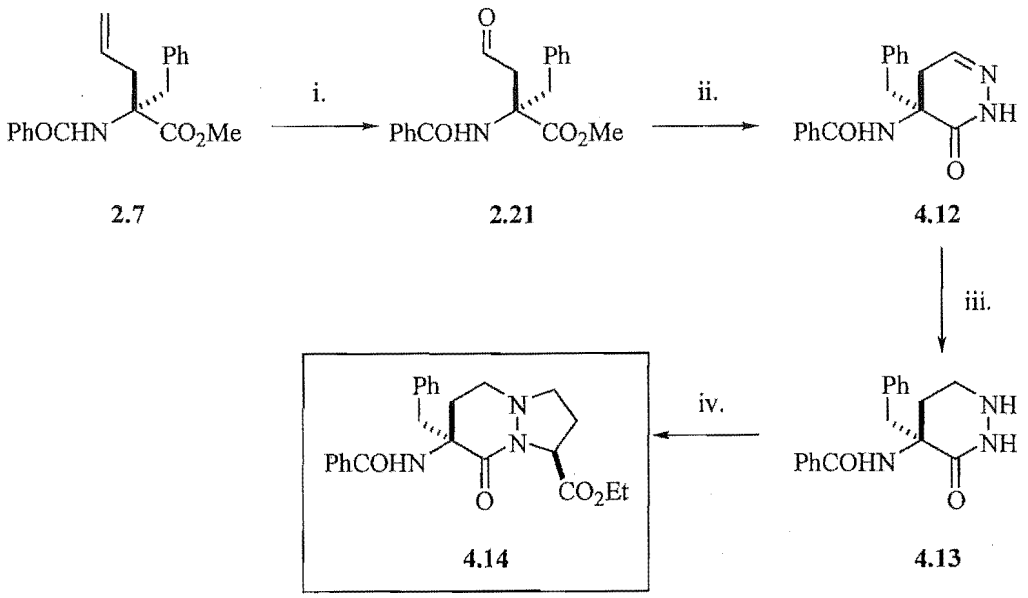


Scheme 4.3. *Reagents and Conditions*: i. NaOH, then PhCHO, CH_2Cl_2, reflux, then PhCOCl, CH_2Cl_2, -20° C, then 0° C for 3 days, 62%; ii. LiHMDS, -78° C, THF, allyl bromide, 93%;, iii. NaOH, MeOH, reflux, quant.; iv. CH_2N_2, 99%.

As the enantiomeric purity of **(-)-2.7**, and hence 2.5, had also previously been determined to be >95% (via synthesis of Mosher esters, see Chapter 2.6), we felt confident it could be used here for the synthesis of a single enantiomer of **4.10**, albeit *N*-benzoylated.

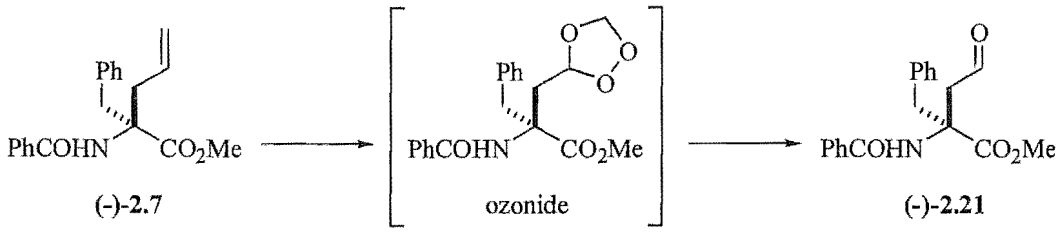
The *N*-benzoyl protecting group was chosen for ease of access of the starting oxazolidinone **2.4**, and to allow synthesis of the required *D*-enantiomer of **2.7**. It should be noted, however, that Cbz-protected oxazolidinones, with the ring substituents *syn*, can also be prepared,[14-16] to allow synthesis of the opposite enantiomer should this be desired. The starting *trans*-phenyl-5-oxazolidinone **2.4** was prepared, in three steps, from (*S*)-phenylalanine as previously described (refer chapter 2),[16] in 62% overall yield. Deprotonation of **2.4** at C-4, with lithium hexamethyldisilaside, and alkylation of the resulting anion with allyl bromide, gave the alkylated oxazolidinone **2.5**, as a single diastereoisomer by ^{1}H NMR, in excellent yield (93%). Note that the stereochemistry at C-4 has been inverted to give the desired *D*-configuration. Subsequent hydrolysis of the oxazolidinone ring of **2.5**, with NaOH in methanol, led to the isolation of the free acid **2.6**, which, on addition of an excess of diazomethane, gave optically active **2.7**, in excellent overall yield (91%, 2 steps).

We next set about preparation of the heterocyclic core of the dipeptide mimetic in a manner similar to that previously reported for racemic **4.10** (see Scheme 4.2). The synthesis of the *N*-benzoyl derivative **4.14** is outlined in Scheme 4.4.



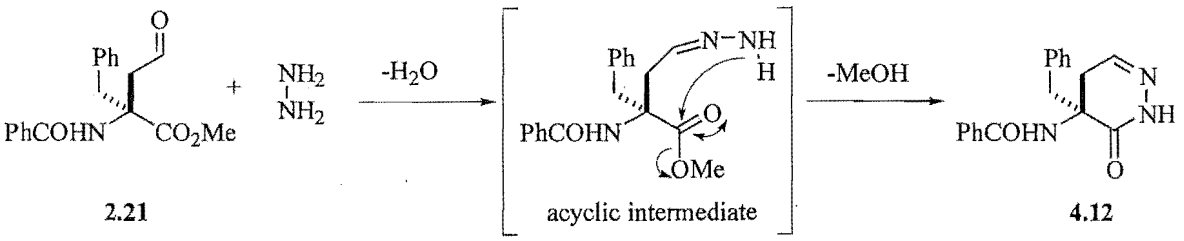
Scheme 4.4. *Reagents and Conditions*: i. O_3, $NaHCO_3$, CH_2Cl_2 / MeOH then Me_2S; ii. hydrazine, THF, reflux, 3 days; iii. Pt_2O (cat.), H_2, MeOH, rt, 48 h or $NaB(CN)H_3$, MeOH / HCl, 0° C to rt, 18 h; iv. CH_2O, ethyl acrylate, reflux.

Firstly, ozonolysis of **2.7** was carried out. A mixture of the olefin and solid $NaHCO_3$ dissolved in a 3:1 mixture of CH_2Cl_2 / methanol, was cooled to -78° C and ozone bubbled through the solution until a deep blue colour resulted. Decomposition of the intermediate ozonide with dimethylsulfide, followed by filtration, gave the previously synthesised aldehyde **2.21**, as a colourless oil, in 96% yield (Scheme 4.5, see also Scheme 2.13). This compound has previously been prepared in the synthesis of the corresponding Mosher esters, in which the enantiomeric purity of **2.7** was established to be >95% (see Chapter 2.6).



Scheme 4.5. *Reagents and Conditions*: O_3, $NaHCO_3$, THF, -78° C, then Me_2S, rt, 6 h, 96%.

Conversion to the cyclic hydrazone was then undertaken, with **2.21** refluxed with hydrazine, in THF, for 3 days, to give **4.12** (Scheme 4.6).



Scheme 4.6. *Reagents and Conditions*: hydrazine hydrate, THF, rt 10 min. then reflux 3 days, 85%.

Previous preparations of the *N*-Boc derivative **4.8**, reported by Boatman *et al*, found that treatment of the aldehyde ester with hydrazine, at room temperature, gave mixtures of the desired cyclic hydrazone along with a number of acyclic hydrazones.[5] TLC and NMR evidence indicated rapid formation of the acyclic hydrazone, and slow formation of the heterocycle. Subsequently, it was found that heating the reaction mixture for an extended period gave the desired cyclic hydrazone in good yield. This was also found to be the case in the preparation of **4.12**, with isolation, after purification, giving a white solid in 85% yield. Measurement of the optical rotation gave an $[\alpha]_D$ = -128.4°, c=1.0 $CHCl_3$.

Hydrogenation of **4.12** over Adam's catalyst (PtO_2), as reported for the preparation of racemic **4.9**,[5] gave variable results in our hands. However, reduction of **4.12** with sodium cyanoborohydride gave the desired tetrahydropyridazinone **4.13**, cleanly, in 73% yield (Scheme 4.7). Measurement of the optical rotation gave $[\alpha]_D$ = -25.7°, c=1.0 $CHCl_3$.

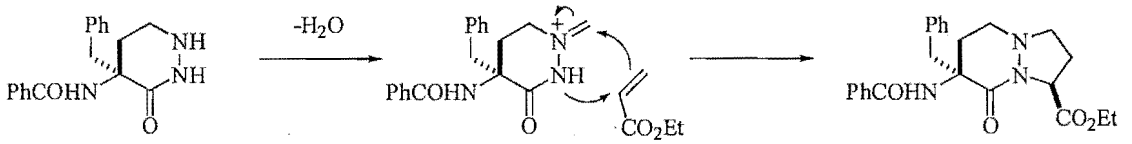
4.12 **4.13**

Scheme 4.7. *Reagents and Conditions*: $NaBH_3CN$, HCl, MeOH, 73%.

Preparation of the core compound **4.13** represents a key result, as it describes the first enantioselective synthesis of a phenylalanine-based tetrahydropyridazinone of this type. This can now be used in the enantioselective preparation of the diazobicyclic template **4.14**.

4.3 Synthesis of the Diazobicyclic Template

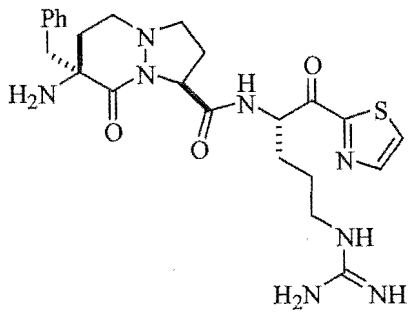
Finally, treatment of **4.13** with formaldehyde, and subsequent heating with an excess of ethyl acrylate, gave rise to a 1,3-dipolar cycloaddition as reported for the preparation of **4.10**.[5] Purification by chromatography gave the desired diazabicyclic template **4.14** in a modest yield of 27% (Scheme 4.8).



4.13 **4.14**

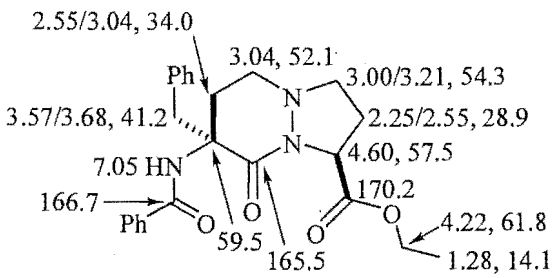
Scheme 4.8. *Reagents and Conditions*: CH_2O, ethyl acrylate, reflux, 4 h, 21%.

The absolute configuration of **4.14** was assigned, as shown, on the basis of the previously determined absolute configuration of oxazolidinone **2.5** (see Scheme 4.3 and Chapter 2), and the relative configuration of template **4.10** as reported by Takahashi and Kahn (see Scheme 4.2).[5,7,8] X-Ray crystallography of derivative **4.15** bound to thrombin, prepared by these researchers from racemic **4.10**, as a potential thrombin inhibitor, also confirmed these assignments.



4.15

This assignment of relative configuration is consistent with that reported for related compounds.[4,17] The ^{1}H and ^{13}C chemical shift data for **4.14**, along with the molecular connectivity information obtained from HSQC and CIGAR experiments, are shown in Figure 4.3.[a]



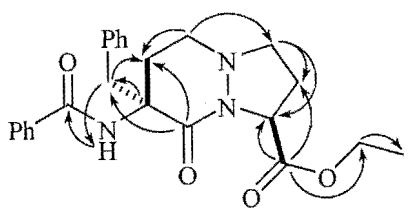


Figure 4.3. Assignment of 1H asnd 13C MNR data for **4.14** based on HSQC and CIGAR derived information.

The factors governing the stereochemical and regiochemical outcomes of 1,3-dipolar addition reactions are still less than clear. However the stereochemical outcome in the preparation of **4.14** can, in some respects, be rationalised when considering the conformation adopted by the tetrahydropyridazone precursor **4.13**. X-Ray crystallography of a number of pyridazinones, and the related cyclic amides,[18] has revealed that these compounds adopt a half chair conformation in the solid state. In these structures, the ring bound amide is essentially planar due to it exhibiting partial double bound character. It is

[a] A 2D NOESY experiment gave no useful information regarding the relative stereochemistry.

thought that this planar character is accentuated upon addition of formaldehyde to form the intermediate imine. Therefore 1,3-dipolar addition, in the presence of ethyl acrylate, can take place with the electrophile approaching from either face of the imine intermediate. Modelling studies of this intermediate, and indeed analysis of the X-ray structures of template derivatives such as **4.15**, indicate that the benzyl group substantially blocks one face of the dipole, such that the favoured approach of the electrophile is from the side opposite this substituent. In the formation of template **4.14**, 1,3-dipolar addition proceeds with the regioselection shown, with the exo product being favoured (Figure 4.4).

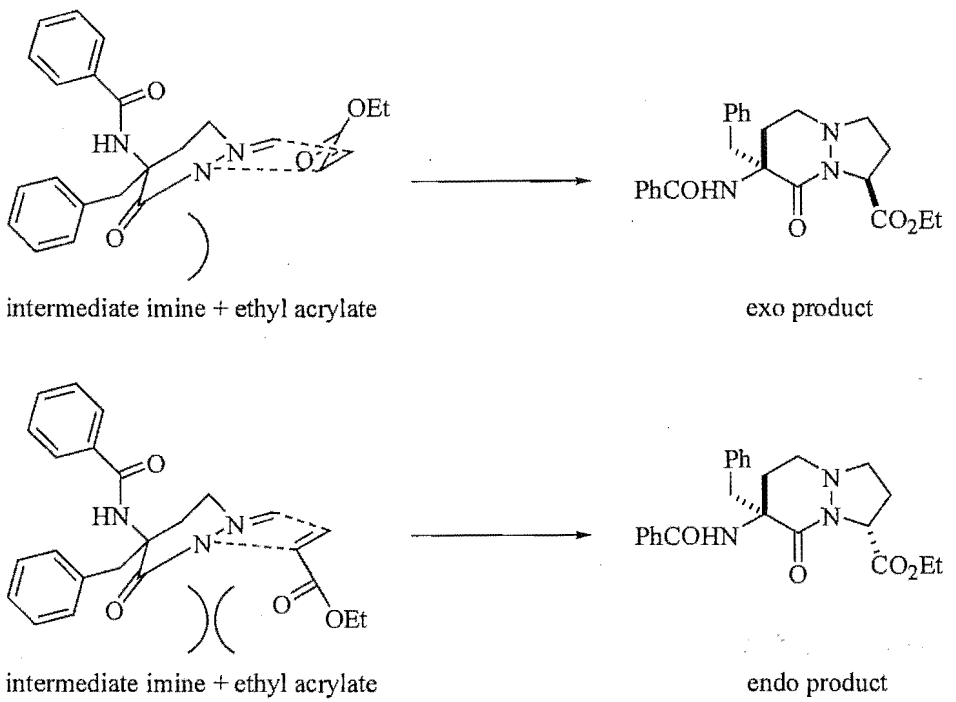
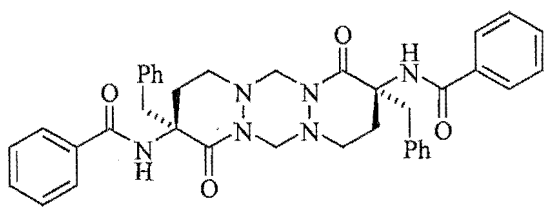


Figure 4.4. X-Ray crystallographic, and modelling, studies suggest that the benzyl substituent blocks one face of dipole intermediate, thereby favouring the formation of the exo-product **4.14**, with stereochemistry shown.

4.3.1 Formation of a Dimeric Byproduct

During initial efforts at inducing 1,3-dipolar cycloaddition to form template **4.14** an interesting byproduct was isolated. It was found that if, on addition of formaldehyde to **4.13** in ethyl acrylate, insufficient heat was applied to promote formation of the desired

template (i.e. 80-90° C instead of reflux at 100-110° C), a single compound could be isolated cleanly as a solid in a moderate yield of 45%. Since 1,3-dipolar cycloadditions of azomethine imines in solution are known to suffer from many side reactions, including dimerisation due to their high reactivities, we were intrigued as to what this byproduct might be. Analyse of the ^{1}H NMR spectrum revealed a resonance pattern similar to that of the starting pyridazinone, but with an additional pair of diastereotopic methylene signals at δ 4.42 and 5.09 ppm. It was thought that we had perhaps isolated a reduced imine, or cyclopropyl derivative of **4.13**, as a result of its reaction with formaldehyde. Further analysis by ^{13}C NMR spectroscopy seemed to support this, with the presence of an additional carbon signal, at δ 64.5 ppm, being observed in the spectrum. However, upon analysis by electrospray mass spectrometry it was revealed that the parent ion (M^{+}) had a mass of 643. This indicated that a dimeric compound had been obtained, with ^{1}H NMR and ^{13}C NMR analysis indicating that it was symmetrical in nature. Further 2-D NMR analysis, in the form of COSY, HSQC and CIGAR experiments, led to the structure being assigned as **4.16**.



4.16

It was concluded that insufficient heat had been applied to the reaction to promote five-membered ring formation upon 1,3-dipolar cycloaddition with ethyl acrylate. Instead, dimerisation, also via 1,3-dipolar addition, had taken place, forming a more stable six-membered ring between two formylated derivatives of **4.13**. The mechanism of formation of **4.16** is suggested in Figure 4.5, with two molecules of the azomethine imine intermediate reacting together via 1,3-dipolar addition to form a central, thermally stable, six-membered heterocyclic ring.

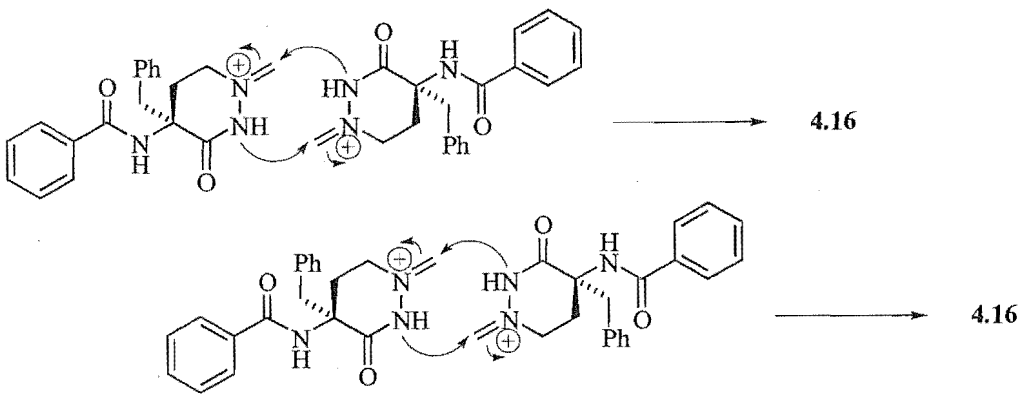


Figure 4.5. Suggested mechanism of formation of the symmetrical dimmer **4.16**

4.4 Conclusion and Future Work

In conclusion, we have developed the first enantioselective synthesis of the phenylalanine-based tetrahydropyridazinone **4.13**, and its conversion to the 2-oxo-1, 6-diazobicyclo[4,3,0]nonane-9-carboxylate dipeptido-mimetic scaffold **4.14**, an important component of extended β-strand mimetics. The nature, and absolute configuration, of the starting amino acid can be varied to give a versatile and general method for the preparation of compounds of type **4.1**. This template has shown versatility in the development of inhibitors for a wide range of serine proteases, in particular that of thrombin. An example is MOL144, a compound that exhibits potent thrombin inhibitory properties as well as good bioavailability.

Key to this synthesis was the preparation of enantiomerically pure samples of the α,α-disubstituted amino acid **2.7**. Existing methodology, used in the synthesis of the racemic template **4.10**, was then employed to allow the preparation of tetrahydropyridazone **4.13**, as a single enantiomer. Subsequent imine formation, and 1,3-dipolar cycloaddition in the presence of ethyl acrylate, led to the desired diazabicyclic template **4.14** being isolated, as a single diastereomer, in moderate yield. The stereochemistry of this compound was

assigned based on the previously determined absolute configuration of **2.5**, and also the relative configuration of **4.10** as reported in the literature.

In addition, it was found that insufficient heating during this 1,3-dipolar cycloaddition reaction led to the formation of an unknown byproduct. Subsequent NMR and mass spectroscopy studies revealed this to be the symmetrical dimer **4.16**, formed as a result of 1,3-dipolar cycloaddition between two imine intermediates of **4.13**.

Future work could include the synthesis of a range of thrombin inhibitors incorporating the diazobicyclic template **4.14**. Variation of Y groups alluded to in Table 4.1, could lead to more potent, and more selective, inhibitors of this important enzyme. In addition, the nature and configuration of the starting amino acid could be varied to establish a general preparation of compounds of type **4.1**. Peptidomimetics containing these templates, and incorporating key residues specific to other enzyme substrates, could be used to probe for potentially new inhibitors of these enzymes.

4.5 References for Chapter Four

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CHAPTER FIVE

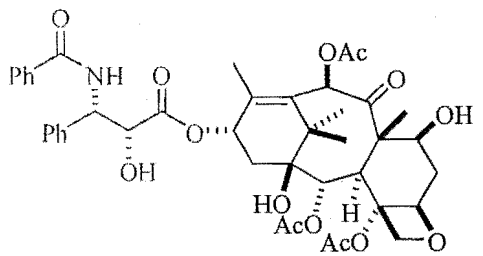
SYNTHESIS OF α-SUBSTITUTED CYCLOHEXENYL-BASED β–AMINO ACIDS BY RING–CLOSING METATHESIS

5.1 Introduction

Recently, there has been increasing interest in the synthesis of cyclic β-amino acids for use in the preparation of β-peptide foldamers with stable and defined conformations. Here we present a versatile ring-closing metathesis (RCM) approach to cyclic β-amino acids that allows the preparation of examples that are either unsubstituted, or substituted, at the α-position.

5.1.1 β-Amino Acids

Although not as abundant as their α-analogues, β-amino acids have recently emerged as an increasingly important class of compound in medicinal chemistry. β-Amino acids are found in humans, animals, microorganisms, marine organisms, in either free form or as derivatives, and possess a wide range of pharmacological properties. They are also found in a number of peptide natural products that exhibit antibiotic, antifungal as well as cytotoxic activity. An example is the α-hydroxy-β-amino acid component of the medicinally important natural product paclitaxel (Taxol®),[1-4] isolated in the mid 1960's from the stem and bark tissues of the Pacific yew tree (*Taxus brevifolia*) (Figure 5.1),[5-7] and currently used in the treatment of a variety of cancers. The intact paclitaxel molecule is required for optimal cytotoxicity, with the stereochemistry of the α-hydroxy-β-amino acid moiety of critical importance.[5]



Paclitaxel (Taxol)

Figure 5.1. β-Amino acids are found in a variety of natural products including the potent anticancer agent placitaxel (Taxol®).

β-Amino acids are important components of β-lactams,[8,9] a class of compound that includes antibiotics,[10] β-lactamase inhibitors,[11] human leukocyte elastase inhibitors,[9] and cholesterol uptake inhibitors.[12] Key examples are the historically important penicillins **5.1** (Figure 5.2),[8,10] with the rapid increase in penicillin-resistant strains of bacteria in recent decades leading to new β-lactam ring systems being discovered and developed. These include the penems **5.2**,[13] carbepenems **5.3** – **5.5**,[14,15] and the so-called third generation antibiotics such as the cephams **5.6**,[16] carbecephams **5.7**,[17] and oxaisocephams **5.8**.[18] Much effort has gone into the synthesis of β-amino acids for use in the development of new and important compounds of this type.

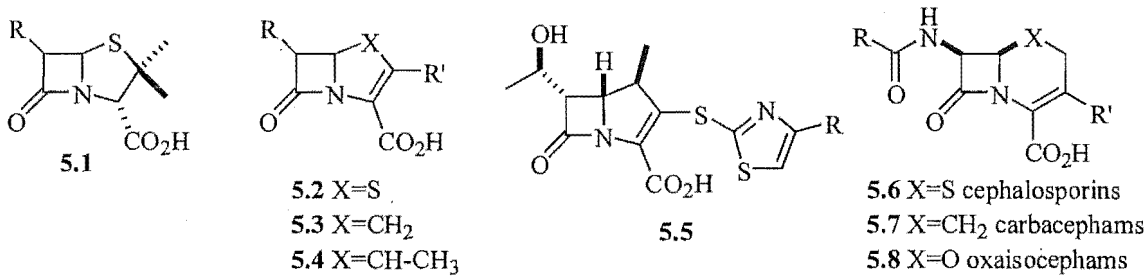
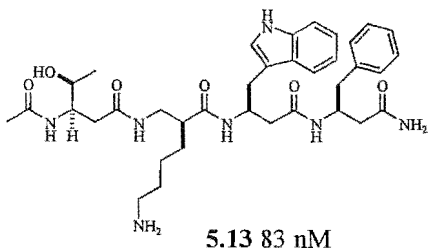
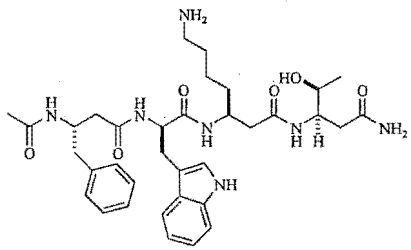


Figure 5.2. β-Amino acids have been used as important precursors to β-lactams.

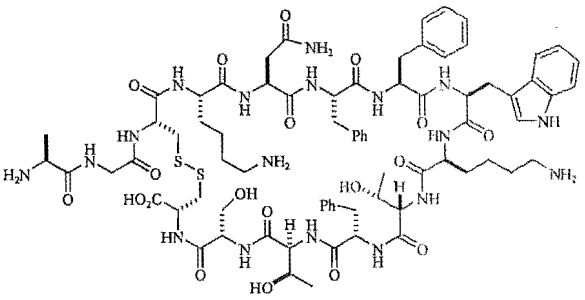
Recently, the development of linear β-peptides for use as therapeutics has resulted in a number of promising finds. Short chain β-peptides have so far been shown to inhibit an intestinal membrane bound cholesterol- and lipid-transporting protein,[19] while others have been shown to possess antimicrobial and haemolytic activities.[20-24] Examples include the peptidase resistant tetrapeptides **5.13** and **5.14** (Figure 5.3), both of which have exceptionally high affinities for a human somatostatin receptor.[25] This was one of the first cases where an α-peptide hormone was shown to be mimicked by a small β-peptide, with **5.14** having emerged as one of the most tightly binding ligands known for this receptor.



5.13 83 nM



5.14 23 nM



Somatistatin, $SRIF_{14}$ 4 nM

Figure 5.3. Tetra-β-peptides **5.13** and **5.14** have been shown to have extremely high binding affinities for a human somatostatin receptor.

β-Peptides also possess enhanced biological stability towards a wide range of proteolytic enzymes, both in *vitro* and in *vivo*, compared with that of their α-peptide equivalents, making them of particular interest in this regard.[26-28]

In addition to linear β-peptides, cyclic peptides such as **5.15** and **5.16** (Figure 5.4), have been shown to adopt secondary structure,[29] forming cylindrical stacks stabilised by hydrogen bonds between adjacent levels. In the (*S*,*S*,*S*,*S*) tetramer **5.15**, the amide bonds are orthogonal to the ring plane and all point in the same direction. The orthogonal amide bonds of the (*S*,*R*,*S*,*R*) tetramer **5.16** alternately point in opposite directions.

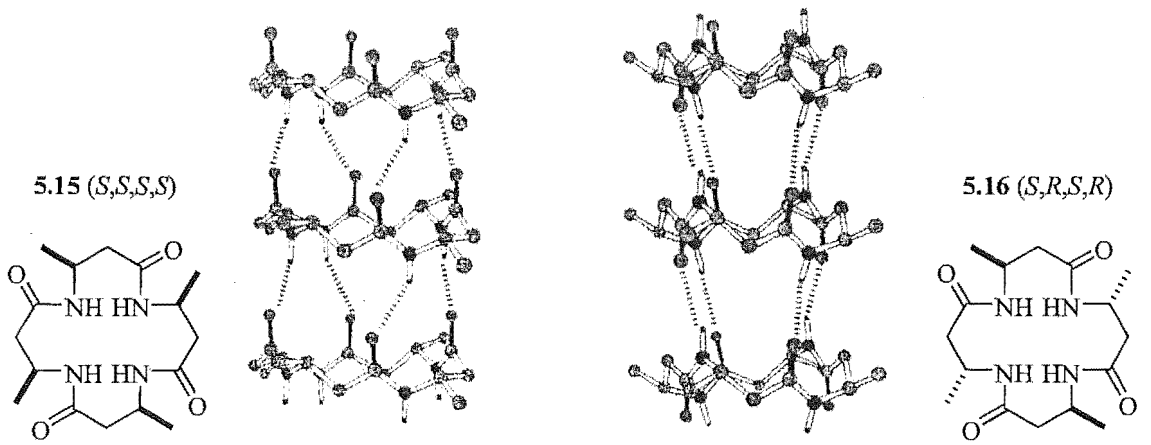
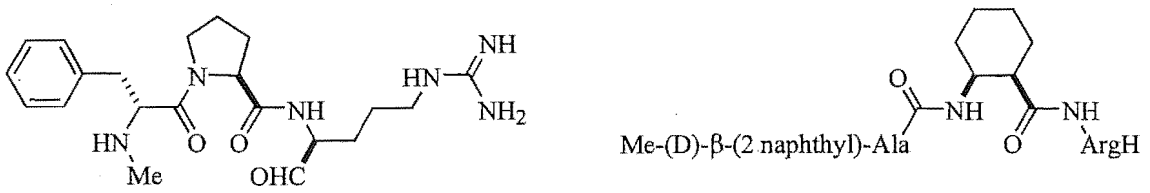


Figure 5.4. Side view of the solid-state structures of the cylindrical stacks composed of the cyclopeptides **5.15** (left) and **5.16** (right).

These cylindrical stacks display similar properties to the peptide nanotubes investigated by Ghadiri *et al*,[30] and Sun and Lorenzi.[31] Compounds of this type are of interest in the development of potential pharmaceuticals as ion pore channels and structural scaffolds, as well as in the area of pseudo-polymer materials research. Similar cyclo-β-peptides have also been shown to have a high affinity for a range of biological receptors,[32] as well as antiprolific activity against the growth of a number of human cancer cell lines.[33]

The chemistry and pharmacology of β-amino acids has been widely reviewed, however the study of cyclic β-amino acids has received somewhat less attention. In addition to their own pharmacological activity, cyclic β-amino acids are useful as building blocks for the preparation of a range of biologically active peptides. Insertion of cyclic β-amino acids in place of α-amino acids, in a biologically active peptide, can lead to increased activity, associated with stabilisation of a particular bioactive conformation, or enhanced bio-stability brought about by the increased resistance of β-peptides to enzymatic degradation.[34,35] An example is the replacement of proline in GYKI 14766 (Figure 5.5), with *cis*-2-aminocyclohexane carboxylic acid (ACHC), to give a series of potent and selective thrombin

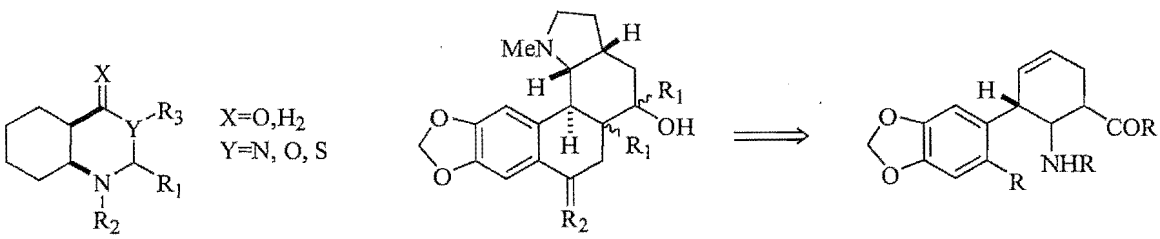
inhibitors. Further extension of the *N*-terminal side-chain led to the development of the more active, and much more selective, inhibitor **5.9**.[36]



GYKI 14766 **5.9**

Figure 5.5. Replacement of α-amino acids with β-amino acids can lead to new and important derivatives, i.e. the potent and selective thrombin inhibitor **5.9**.

In addition, cyclic β-amino acids are used as starting materials for the preparation of a wide range of heterocycles, potential pharmaceuticals, and natural products, with enantiomerically pure forms also serving as effective chiral auxiliaries or additives. Heterocycles of type **5.10** (Figure 5.6), have been shown to exhibit excellent anti-inflammatory activity,[37] while more complex alkaloids such as clivonine, clividine, and gelsimine, make use of cyclic β-amino acids as key precursors.[38,39]



5.10 Clivonine

Figure 5.6. Examples of heterocyclic compounds synthesised from cyclic β-amino acids

Early syntheses of β-lactam antibiotics utilised cyclic β-amino acids to generate a β-lactam core with the desired stereochemistry. An example is thienamycin **5.11** (Figure 5.7), an early carbapenam, whose wide spectrum of antibacterial activity triggered intense synthetic interest in this area.[40,41]

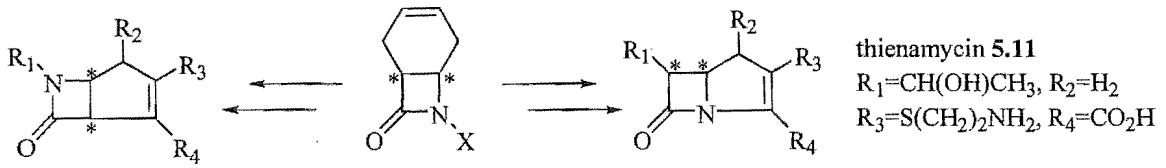
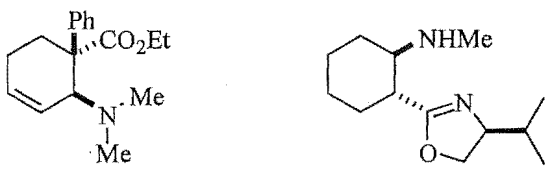


Figure 5.7. Cyclic β-amino acids are important precursors to β-lactam antibiotics such as thienamycin **5.11**.

Synthetic examples of cyclic β-amino acids include the α-substituted ACHC derivative Tilidine (Figure 5.8).[42] This compound was synthesised via a 1,4 Diels-Alder cycloaddition reaction to give a mixture of isomers, the minor of which is currently in clinical use for the treatment of moderate to severe pain.



Tilidine **5.12**

Figure 5.8. Tilidine is an example of an α-substituted ACHC, while **5.12** has been used in the area of asymmetric catalysis.

This Diels-Alder strategy has also been recently utilized by Wipf *et al* for the preparation of ring-functionalised ACHC's for use as new ligand scaffolds to aid in asymmetric transformations. Compound **5.12**, is an ACHC derivative used in catalytic asymmetric alkylzinc additions to aldehydes.[43,44]

5.1.2 β-Peptides

Recently, an exciting, and pioneering, new chapter has been opened in the area of β-amino acid research. Early investigations of polymeric β-peptides indicated that they were able to adopt stable helical structures,[45-47] with later studies revealing that sheet structures and turns can also be adopted. Seebach *et al*, along with researchers from Novartis Pharma A. G.,

showed that a range of acyclic β-oligopeptides adopt a distinct helical structure in solution. Subsequent research has revealed that variously substituted β-peptides, where the nature and position of the side-chains were varied, also adopt the same type of helix and that the nature of the substituent influences the precise nature of the folding. It was further found that as few as 4-6 residues are required to induce this form of secondary structure, a stark contrast to α-peptides, where upwards of 15 to 20 amino acid residues are necessary before distinct secondary structure can be exhibited. Figure 5.9 illustrates the various types of helical conformations that can be adopted by polyamide sequences composed of C^2- and/or C^3-substituted β-amino acids.

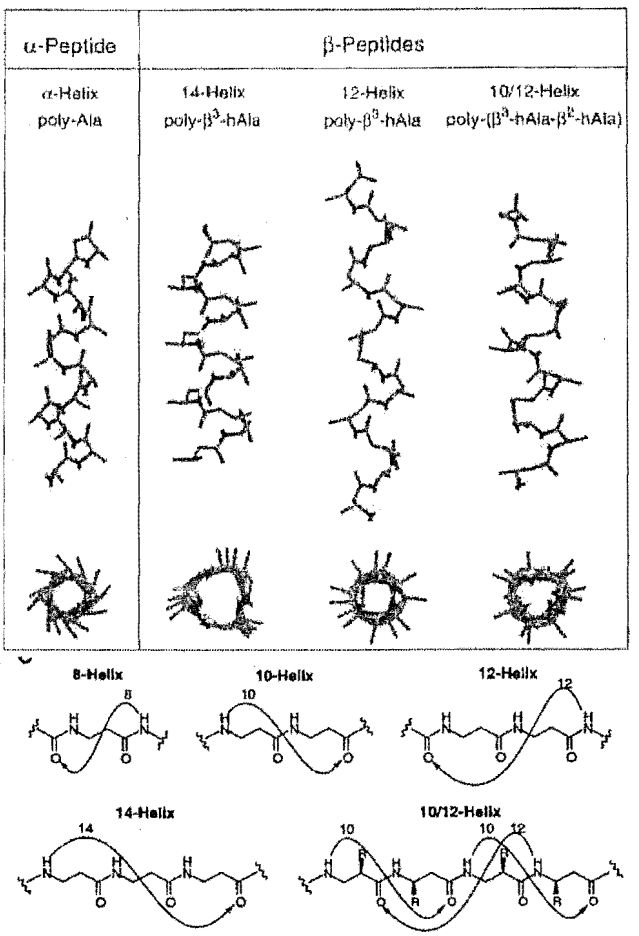


Figure 5.9. Structures of the α-helix, β-peptide-based 14-helix, 12-helix, and 10/12-helix.[48]

In addition, helices formed by β-peptides were not only stable at room temperature, but remarkably resistant to unfolding at higher temperature.[49] While eventually being made to unfold, β-peptides of this type were quickly observed to recover their helical form spontaneously and rapidly. This type of behaviour is seen as desirable in an oligomer that is of interest primarily for its predictable and reproducible folding patterns and has led to the development of helical β-peptides as potential pharmaceuticals. Compounds of this type are also of interest in such areas as protein recognition research, pharmaceuticals, and the development of DNA-binding proteins where recurring helical structures interact directly with the major groove of DNA. In all these areas the nature of the secondary structural interactions play a key role in the biological process.

The conformation of β-peptides can be analysed in terms of the main chain torsional angles, which are assigned the angles ω, ϕ, θ, ψ (Figure 5.10). Folded helical or turn-like conformations of β-peptides require a gauche conformation about the θ torsion angle defined by the C^2-C^3 bond. A *trans* rotamer leads to a fully extended conformation provided the values of ϕ and ψ are appropriate. The effects of substituents on the conformation of β-amino acids have been the subject of extensive experimental studies, and are summarised in Figure 5.12.[50-53]

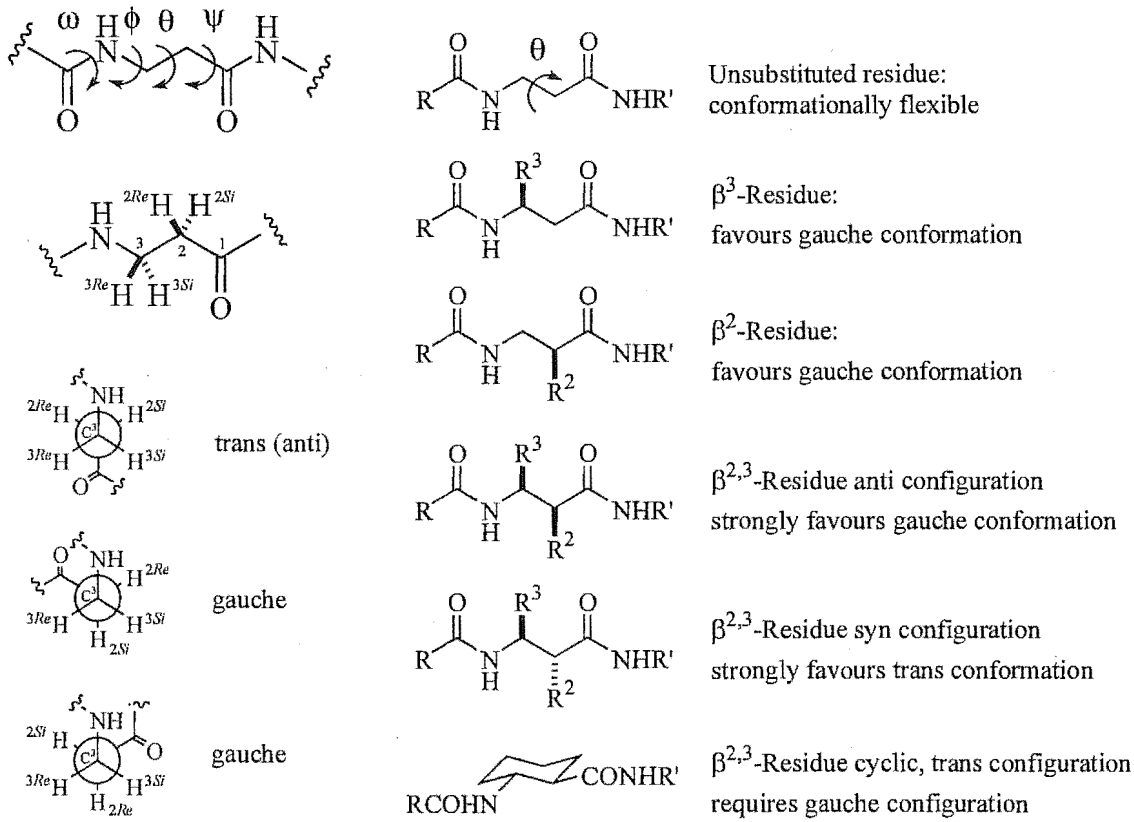


Figure 5.10. Effects of substituents on the torsion angle θ.

The unsubstituted β-amino acid, β-alanine, is highly flexible, analogous to Gly in the α-amino acids, with alkyl substituents at positions 2 and 3 favouring a gauche conformation about the C^2-C^3 bond. C^2, C^3-Disubstituted amino acids are even more conformationally constrained and favour gauche conformers when the substituents are *anti*. Gauche-type torsion angles are even more strongly promoted when these atoms are included in a cycloalkane ring, such as in *trans*-2-aminocyclohexanecarboxylic acid (ACHC). Gellman *et al* demonstrated that a hexamer composed of a repeating unit of *trans*-2-aminocyclohexanecarboxylic acid (*trans*-ACHC), led to extremely stable helical structures where the cyclohexyl rings adopted a step-like arrangement along the backbone (Figure 5.11).[54,55]

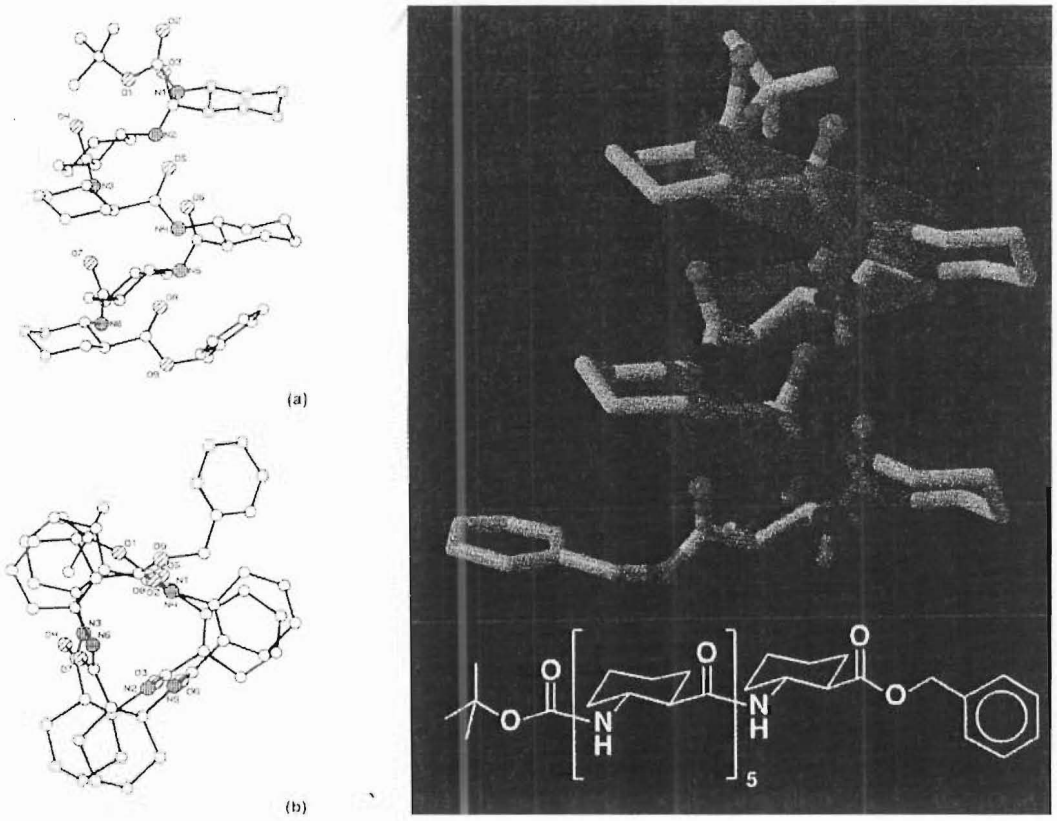
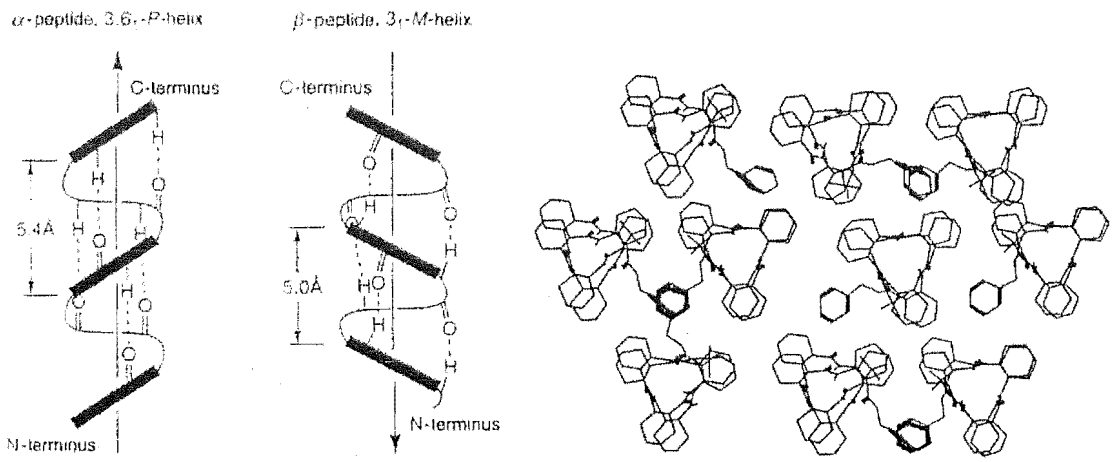


Figure 5.11. β-Peptide hexamer with *trans*-2-aminocyclohexanecarboxylic acid (ACHC) repeating units adopts a helical structure (ribbon) in the solid state.[54]

Gellman predicted, and showed experimentally, that these helices were 14-helices, i.e. stabilised by 14-membered hydrogen-bonded rings, and differed from that of the α-helix in many respects. The 14-helix adopted by the hexamer assumes a more compressed helical structure, having a slightly wider radius, 2.7Å, and a pitch of 5.0 Å, compared with for that of the α-helix (2.2Å and 5.4 Å respectively). It was also found that the amide carbonyl and NH groups project towards the N- and C-terminii, respectively, in the 14-helix, resulting in a net dipole opposite to that of the α-helix (Figure 5.12a). Further, while the α-helix has a 3.6-residue repeat, the 14-helix repeats approximately every 3 residues, which positions the side chains of every third residue directly above one another along one face of the helix. (Figure 5.11b, see also Figure 5.9).

It was noted that the *trans*-ACHC based hexamers developed by Gellman had the same backbone conformation and hydrogen-bonding pattern as the 14-helix compounds synthesised by Seebach (Figure 5.9). Further studies showed these cyclic β-amino acid based oligomers have a much higher intrinsic propensity for helical folding than did the acyclic oligomers.



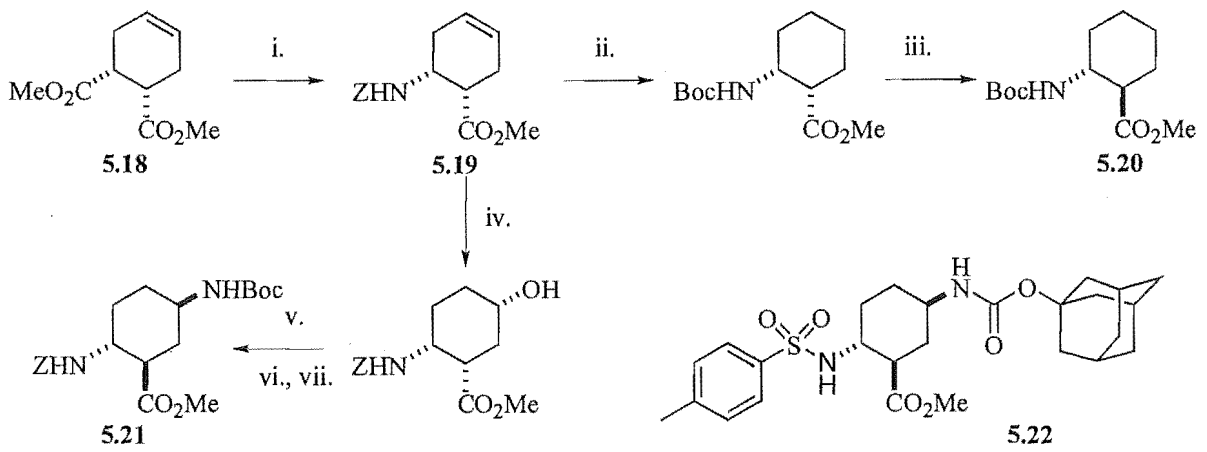
a) b)

Figure 5.12.[55]

One of the most striking properties exhibited by these ACHC hexamers was their packing pattern adopted in the solid state. It was observed that there are two types of three-way interfaces found in this packing pattern, one 'tight' and the other 'loose'. The tight three-way interface, involving extensive cyclohexyl-cyclohexyl contacts, is displayed by the three molecules in the upper left corner of Figure 5.12b. The loose three-way interface, with benzyl groups and solvent at the centre, is displayed by the three molecules in the lower left corner of Figure 5.12b. This packing pattern suggests a strategy for creating β-peptides that adopt a well-defined tertiary structure due to the extensive cyclohexyl-cyclohexyl contacts between adjacent molecules. This has been proposed as a method for developing β-peptides, based on this trimeric cyclohexane packing motif, that adopt a three-helix bundle in solution, where the cyclohexane-cyclohexane contacts are promoted by the hydrophobic effect. The further development of water-soluble β-peptides would require incorporation of hydrophilic residues

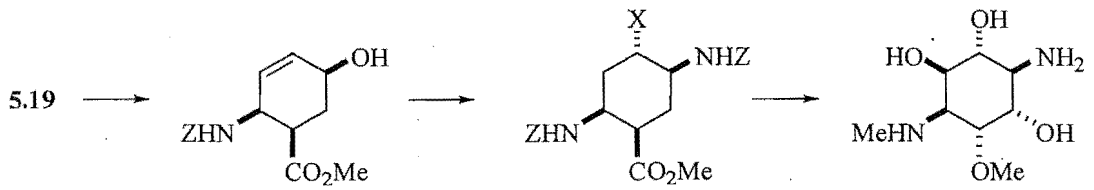
to compliment the hydrophobic *trans*-ACHC residues. As the 14-helix has approximately 3 residues per turn, β-peptides that contain a *trans*-ACHC residue at every third position, with hydrophilic residues at adjacent positions, should adopt helical conformations with a hydrophobic 'stripe' running along one side. The nature of these hydrophilic residues, and their positioning, would potentially determine the properties, and stacking patterns, exhibited by these structures. Conceivably this could lead to the development of helical bundle motifs, based on β-peptides, analogous to that displayed by many transmembrane bound proteins and a number of electron and proton transport proteins. Given that a small number of underivatised *trans*-ACHC residues constrain the β-peptides to adopt a stable defined conformation, a need still exists to explore more highly functionalised versions of these oligomers in an attempt to develop medicinally useful compounds. While a host of cyclic α-amino acids are known, there still remains a clear need to develop a range of cyclic β-amino acids if one is to control the structure, and hence function, of β-peptides derived from them in a predictable fashion.[56] New and versatile methods for the preparation of cyclic β-amino acids, particularly those bearing substituents, are needed to achieve this goal. The synthesis of such compounds represents the pioneering of a major new field of molecular design focussed on synthetic polymers, or foldamers, with protein-like folding properties and protein-like activities.

To date, access to ACHC monomers, and their unsaturated equivalents, for use in synthesis, has predominantly been through use of crystallisation or chemico-enzymatic methods. Nohira *et al* accessed optically active samples via preferential crystallisation of *N*-benzoyl salts.[57] Gellman utilised chemistry developed by Kobayashi *et al*, for the enzymatic desymmetrization, via selective ester hydrolysis, of diester **5.18** (Scheme 5.1), and subsequent selective Curtius degradation, to give *cis*-aminocyclohexenecarboxylic acid **5.19**.[58] Conversion to *trans*-ACHC **5.20** could easily be achieved via hydrogenation and epimerisation. Diamino derivatives such as **5.21** have also been prepared, with the relative, and absolute stereochemistry being verified by its conversion to **5.22**, for which a crystal structure was obtained.[59]



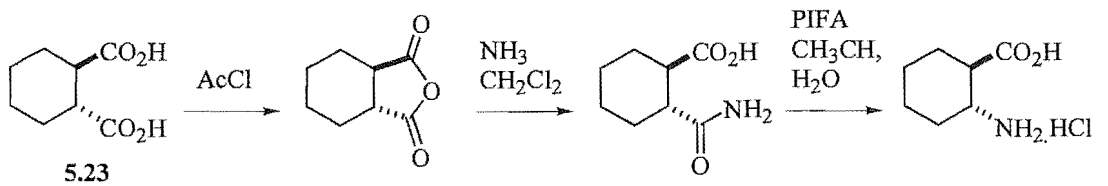
Scheme 5.1. *Reagents and Conditions*: i. a) PLE, 98%, 96% ee b) Curtius rearrangement; ii. a) H_2, Pd-C, MeOH, b) Boc_2O, K_2CO_3, 31% from 5.18; iii. NaOME, MeOH, reflux, 70%; iv. a) CF_3CO_2H, 0° C, quant. b) KI, $NaHCO_3/H_2O$-CH_2Cl_2, rt, 98% c) DBU, benzene, reflux, 94% d) MeI Ag_2O/DMF, rt, 95% e) NaOMe, 0° C, 99%; v. a) MsCl, Et_3N, 0° C, b) $Bu_4N^+ N_3^-$ c) P(n-Bu)$_3$.H_2O d) Boc_2O, 88%; vi. H_2, Pd-C then CbzCl, DIEA, 74%; vii. NaOMe, MeOH, reflux, 60%.

This methodology was originally developed by Kobayashi, for the preparation of aminocyclohexenecarboxylic acid derivatives for use in the enantioselective synthesis of fortamine, the aminocyclitol moiety of the antibiotic fortamicin A.[60]



Scheme 5.2. Enantioselective synthesis of Fortamine.

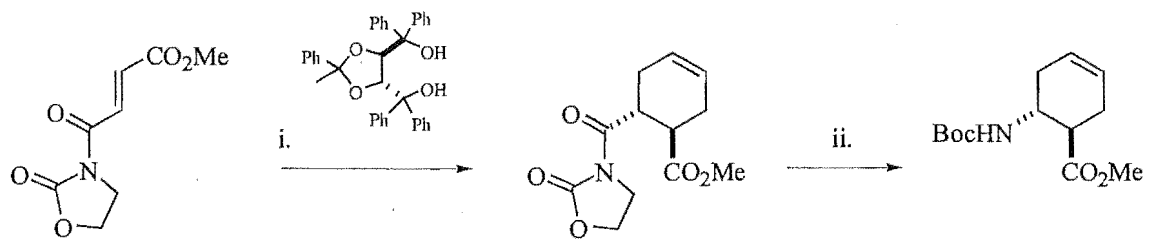
Berkessel *et al* used (*R*)-1-phenylethylamine to selectively crystallise and isolate the optically pure diacid **5.23**. This was then utilised in a one pot-procedure, via the Hofmann degradation of the corresponding anhydride, to give *trans*-ACHC in good yields.[61] Bernath *et al* had previously accomplished this with the equivalent cyclohexene compounds.[62]



5.23

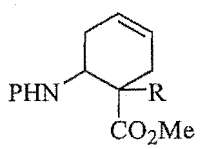
Scheme 5.3. One-pot procedure for preparation of *trans*-ACHC from diacid **5.23**.

Wipf *et al* have adopted a combination of Diels-Alder and chiral auxiliary chemistry to access ACHC's for use in asymmetric catalysis.



Scheme 5.4. *Reagents and Conditions*: i. $Ti(O\text{-}iPr)_2Cl_2$ (10mol%), 4 Å sieves, butadiene, 0° C, 58 h, 97%, 85% ee; ii. a) LiOH, H_2O_2, H_2O/THF, 0° C, 85%, b) DPPA, Et_3N, t-BuOH, 74%.

As such, *trans*-aminocyclohexenecarboxylic acid represents an attractive target towards the development of derivatised cyclic β-amino acids, for use in the synthesis of novel foldamers. To this end, we set out to develop a method whereby a range of cyclic β-amino acids could be synthesised. It was proposed that we use a versatile ring-closing metathesis (RCM) approach to the synthesis of cyclic β-amino acids, that allowed the preparation of compounds of type **5.53**, that were either unsubstituted, or substituted at the α-position.

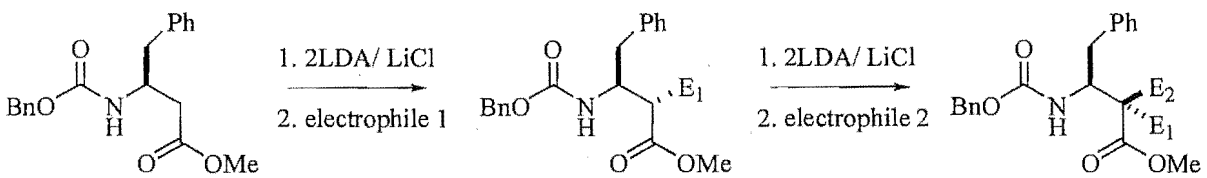


5.53

This second class of compound represents an important addition to the family of cyclic β-amino acids. The olefin of these units is able to be hydrogenated, to give the corresponding saturated analogue, or functionalised to give new and important derivatives.

5.2 Synthesis of α–Free Cyclohexenyl-Based β-Amino Acids

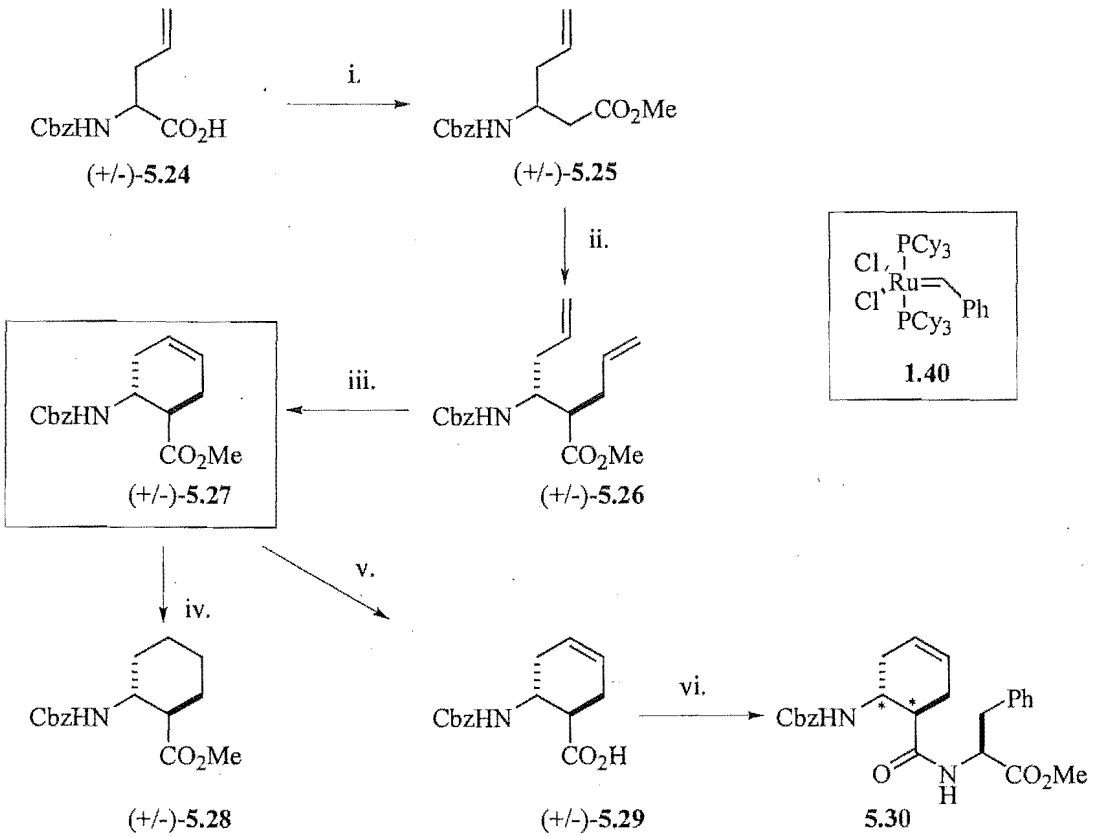
We set out to develop a method whereby both the α-free and α-substituted derivatives could be accessed from a common precursor. The proposed synthesis involves the use of chemistry developed by Podlech and Seebach for the stereoselective alkylation of *N*-Cbz-protected β-amino acids.[63] Here, alkylation of Cbz-protected 3-amino-4-phenylbutanoic acid methyl ester, with a range of electrophiles, via a dilithiated intermediate, led to the formation of the α-substituted derivative as a single diastereoisomer (Scheme 5.5). It was also found that subsequent alkylation of these α-substituted derivatives, in a similar manner, gave rise to the α,α-disubstituted derivative, also as a single diastereoisomer, with the stereochemistry shown.



Scheme 5.5. Stereoselective alkylation of β-amino acids developed by Podlech and Seebach.[63]

These findings led us to consider the use of 3-Cbz-aminohex-5-enoate as a common precursor for the synthesis of cyclohexenyl-based β-amino acids. The basic synthetic strategy involves the selective allylation of 3-Cbz-amino-hex-5-enoate **5.25**, obtained from Cbz-protected allyl-glycine **5.24**, to give a diene that is then cyclized by RCM to give the corresponding unsubstituted aminocyclohexenecarboxylic acid **5.27**. In addition, α-substituted cyclohexenyl β-amino acids can be prepared by an alkylation-allylation sequence, the order of which dictates the stereochemistry of the ring substituents. The synthesis of the α-free *N*-Cbz-

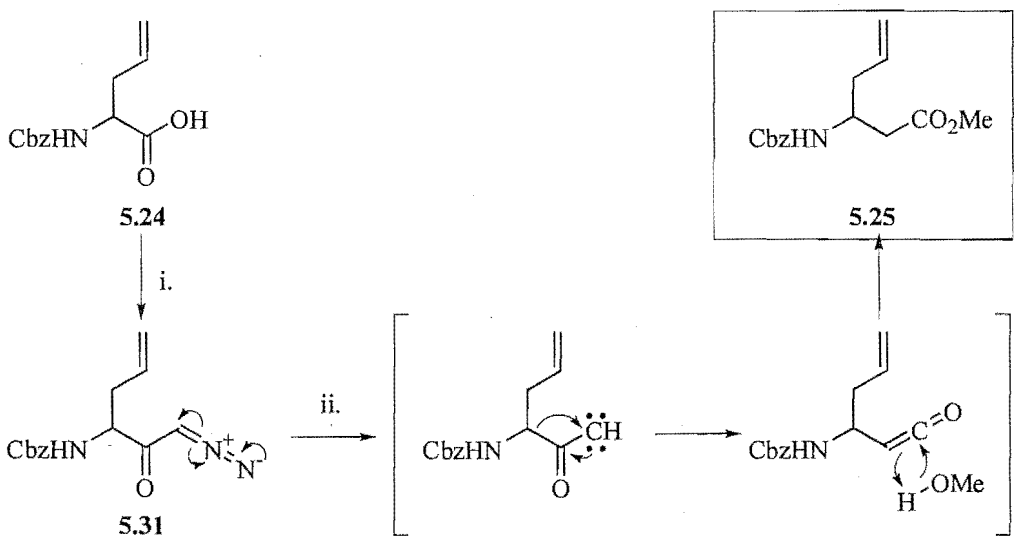
aminocyclohexenecarboxylic acid methyl ester **5.27**, along with some simple derivatives, is outlined in Scheme 5.6.



Scheme 5.6. *Reagents and Conditions*: i. a) Et_3N, $ClCO_2Et$,THF, -15° C, 15min, then diazomethane, 0°; b) AgOBn, Et_3N, MeOH, -25° C to rt; ii. LiCl, 2 equiv LDA, -78° C, THF then allyl bromide; iii. **1.40**, benzene, reflux; iv. H_2, Pd-C, MeOH then DIEA, CbzCl, DMAP, CH_2Cl_2; v. NaOH, MeOH; vi. EDCI, HOBt, DIEA, *L*-PheOMe.HCl.

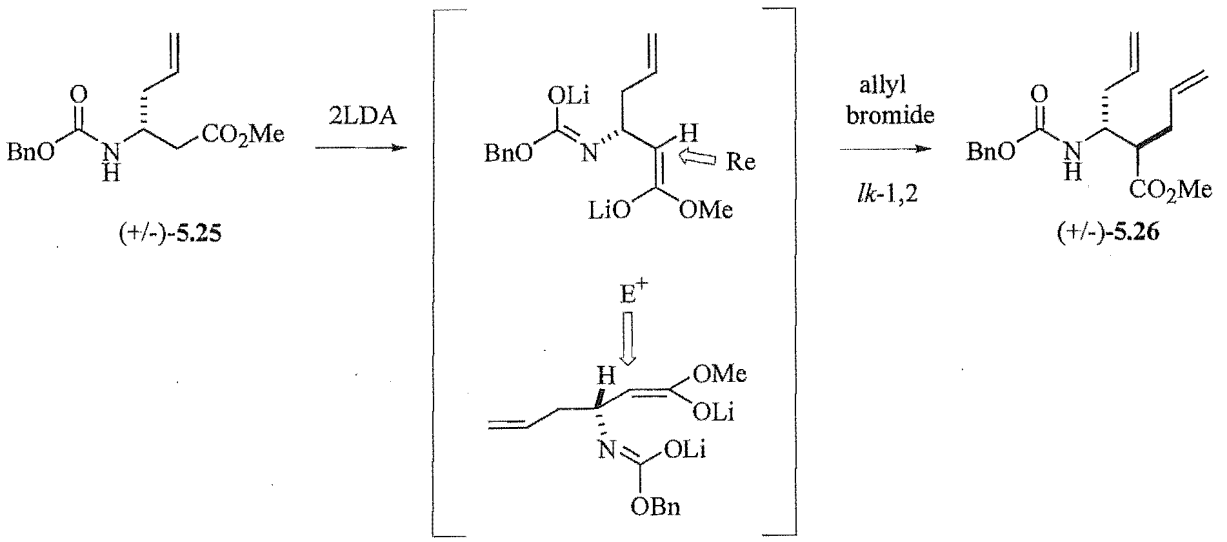
The synthesis began with the preparation of the key 3-Cbz-aminohex-5-enoic acid methyl ester **5.25**. Among the many methods for the synthesis of β-amino acids, we chose to use Arndt-Eistert methodology for its flexibility, high yields and its use of readily available starting materials. Thus, (+/-)-*N*-Cbz-allylglycine **5.24** was reacted with triethylamine and ethylchloroformate, to form the corresponding mixed anhydride, which, upon treatment with diazomethane, gave the diazoketone intermediate **5.31** (Scheme 5.7). A characteristic singlet

at δ_H 5.45ppm, corresponding to the CHN_2 proton, signified the successful preparation of this intermediate which was isolated as a yellow solid in 98% yield. Exposure of the diazoketone **5.31**, to silver benzoate, at low temperature, in the presence of methanol, with the exclusion of light, resulted in a Wolff rearrangement to form the desired (+/-)-3-Cbz-aminohex-5-enoate **5.25**, in 94% yield.



Scheme 5.7. *Reagents and Conditions*: i Et_3N, $ClCO_2Et$, THF, -15° C, 15min, then diazomethane, 0° C; ii) AgOBz, Et_3N, MeOH, -25° C to rt, 94%.

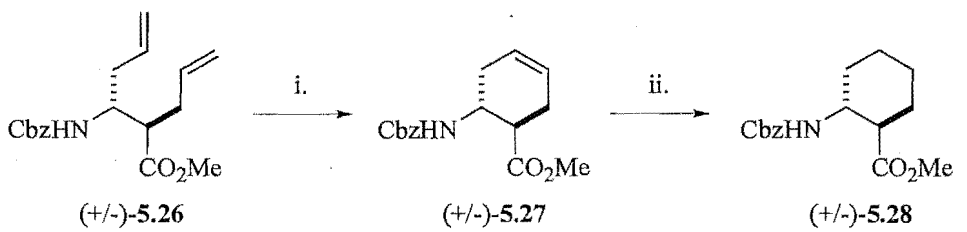
With this key β-amino acid in hand we set about introducing a substituent at the α-position, in the manner previously described by Podlech and Seebach.[63] Thus, **5.25** was deprotonated with LDA, in THF at -78° C, in the presence of LiCl, and the corresponding enolate alkylated with allyl bromide (Scheme 5.8). It is well documented that these conditions proceed via a doubly lithiated intermediate to give the relative stereochemistry shown, with the addition of LiCl leading to higher yields and better diastereoselectivities.[63-66] The mechanism for this reaction is shown in Scheme 5.8.



Scheme 5.8. Stereoselective alkylation of **5.25** via a doubly lithiated intermediate

Here, the bulky benzyloxycarbamide group on the β-carbon effectively blocks one face of the planar dilithiated enolate intermediate. The result is that the incoming electrophile approaches from the *Re* face of the enolate, with the alkylation occurring with relative *topicity lk*-1,2 (*like*-1,2) as shown. Purification by silica chromatography gave (+/-)-**5.26**, as a single diastereoisomer by ^{1}H NMR, in 59% overall yield.

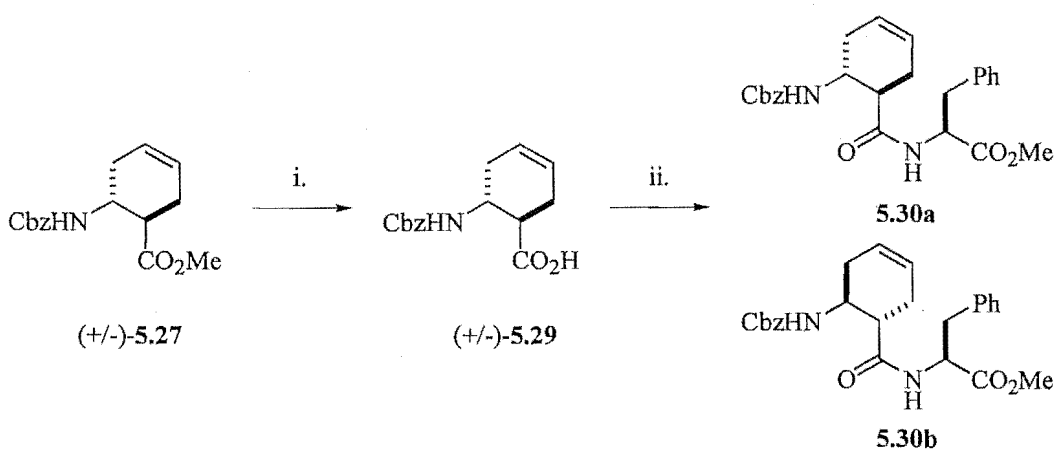
Next, we demonstrated the basic methodology for the preparation of cyclic β-amino acids of this type, by subjecting diene **5.26** to ring closing metathesis conditions (Scheme 5.9).[67]



Scheme 5.9. Reagents and Conditions: i. benzene, rt, 2h, **1.40** 96%, **1.42** 94%; ii. H_2, Pd-C, MeOH then DIEA, CbzCl, DMAP, CH_2Cl_2, 84%.

To this end, diene **5.26** was treated with Grubbs' ruthenium catalyst **1.40**, in *dry degassed* benzene, and stirred at room temperature for 2h. Purification of the residue by silica chromatography gave the cyclic β-amino acid **5.27**, as a single diastereoisomer by ^{1}H NMR, in 96% yield. Comparable results were also obtained using Grubbs' second generation catalyst **1.42**. The *trans* relative stereochemistry of **5.27** was confirmed by its conversion to the known aminocyclohexane carboxylic acid **5.28**. Thus, olefin **5.27** was hydrogenated in the presence of 10% palladium-on-carbon, followed by reprotection with benzylchloroformate, to give the literature compound **5.28** in 84% yield.[59] Comparison of ^{1}H NMR data for **5.28**, with that reported in the literature, confirmed the assigned relative stereochemistry of both the cyclic β-amino acids, as well as that of their dienic precursor **5.26**.

Hydrolysis of the ester of **5.27** in the presence of NaOH, gave the corresponding free acid **5.29** in quantitative yield, a derivative suitable for incorporation into peptides (Scheme 5.10). This was illustrated by the coupling of **5.29** with phenylalanine methyl ester, under standard conditions, to give the dipeptide diastereoisomers **5.30a** and **5.30b**, in a ratio of 1:1 by ^{1}H NMR, in 91% combined yield. However, the epimers could not be separated by silica-based chromatography.

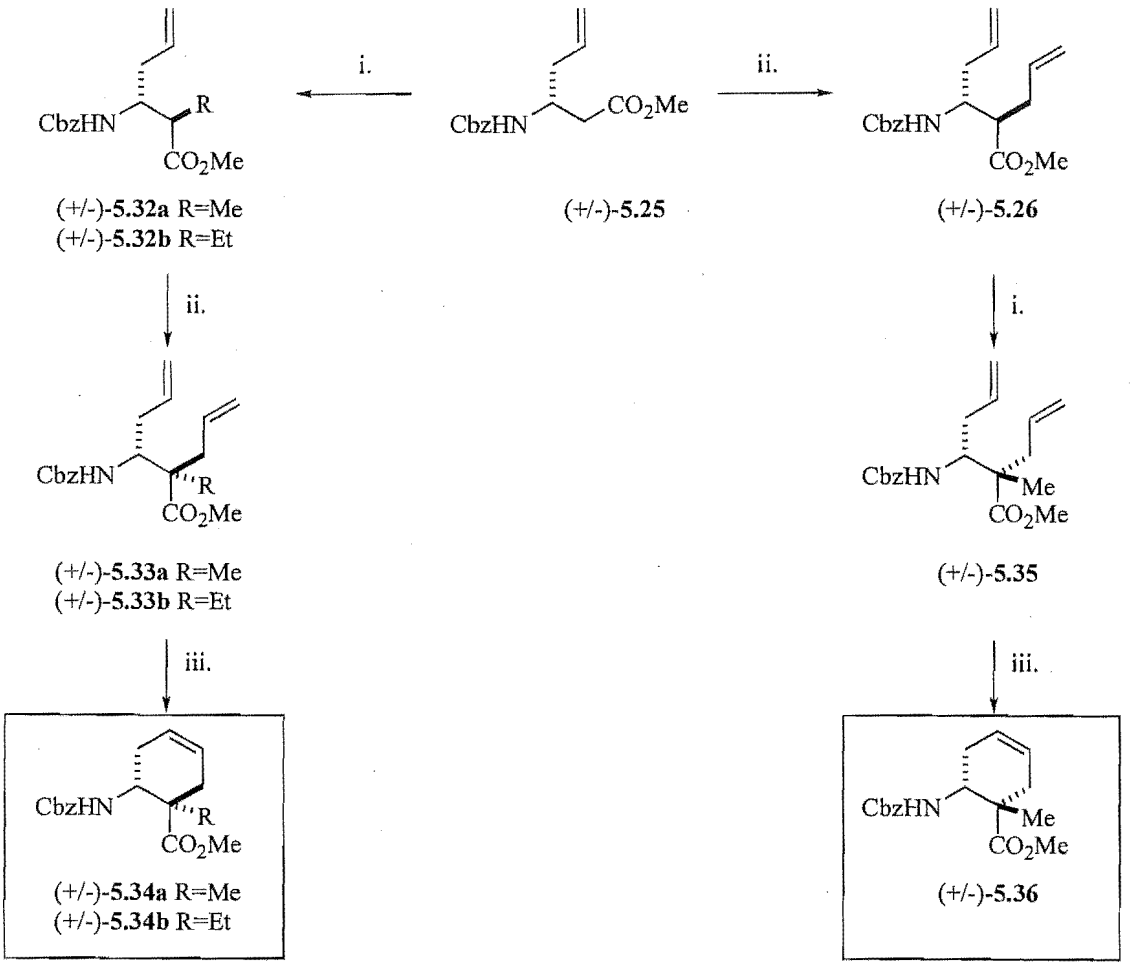


(+/-)-**5.27** (+/-)-**5.29**

Scheme 5.10. *Reagents and Conditions*: NaOH, MeOH, 100%; ii. EDCI, HOBt, DIEA, *L*-PheOMe.HCl, CH_2Cl_2, 91%.

5.3 Synthesis of α-Substituted Cyclohexeneyl-Based β-Amino Acids

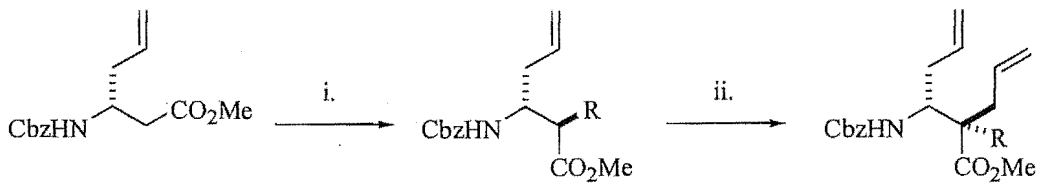
With the basic methodology for the synthesis of α-free cyclohexenyl β-amino acids in place, we turned our attention to the synthesis of α-substituted cyclohexenyl β-amino acids. This strategy involved the preparation of suitable dienes, from the common precursor **5.25**, via an alkylation – allylation sequence, the order of which dictated the stereochemistry of the ring. The synthesis of both *trans*-α-substituted and *cis*-α-substituted cyclohexenyl β-amino acids is shown in Scheme 5.11.



Scheme 5.11. *Reagents and Conditions*: i. LiCl, 2 equiv of LDA, THF, -78° C, MeI or EtI, **5.32a** 86%, **5.32b** 63%, **5.35** 68%; ii. LiCl, 2 equiv of LDA, THF, -78° C, allyl bromide, **5.33a** 47%, **5.33b** 42%, **5.26** 59%; iii. **1.40**, benzene, reflux, **5.34a** 96%, **5.34b** 94%, **5.36** 91%.

The synthesis began with the preparation of the α-substituted *trans* examples **5.34a** and **5.34b**. Alkylation of **5.25** with either methyl or ethyl iodide, in the presence of LDA and LiCl at -78° C in THF, gave the α-methyl and α-ethyl substituted ethyl esters **5.32a** and **5.32b**, after purification by silica chromatography, in 86% and 64% yield respectively. ^{1}H NMR analysis indicated that a single diastereoisomer had being isolated in each case, as for the preparation of **5.26** (Scheme 5.8), with the stereochemistry at C^2 and C^3 as shown.

Compounds **5.32a** and **5.32b** were then alkylated a second time, with allyl bromide, to give, after purification by silica chromatography, the α,α-disubstituted dienes **5.33a** and **5.33b** in 47% and 42% respectively (Scheme 5.12).

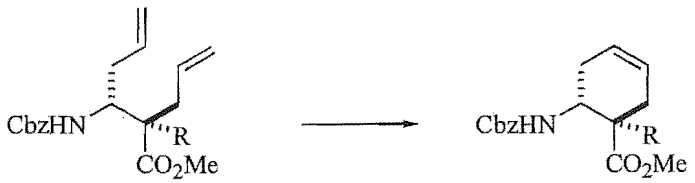


(+/-)-**5.25** (+/-)-**5.32a** R=Me (+/-)-**5.32b** R=Et (+/-)-**5.33a** R=Me (+/-)-**5.33b** R=Et

Scheme 5.12. *Reagents and Conditions*: i. LiCl, 2 equiv of LDA, THF, -78° C, MeI or EtI, **5.32a** 86%, **5.32b** 63%; ii. LiCl, 2 equiv of LDA, THF, -78° C, allyl bromide, **5.33a** 47%, **5.33b** 42%.

Note that this second alkylation leads to an inversion of stereochemistry at C^2, and is the result of the electrophile approaching the planar enolate from the face opposite the benzyloxycarbonylamino group, in a manner similar to that for the preparation of **5.26** (Scheme 5.8). On exposure of **5.33a** and **5.33b** to RCM conditions, this stereochemistry lends itself to a *trans* relationship being adopted by the benzyloxycarbonylamino and methyl ester substituents of the subsequent cyclic β-amino acids. As for the preparation of **5.32a** and **5.32b**, dienes **5.33a** and **5.33b** were isolated as single diastereoisomers by ^{1}H NMR.

Subsequent exposure of dienes **5.33a** and **5.33b** to Grubbs' ruthenium catalyst **1.40**, in benzene at reflux gave, after purification by silica chromatography, the α-substituted cyclic β-amino acids **5.34a** and **5.34b**, each as a single diastereoisomer by ^{1}H NMR as shown, in 96% and 94% yield respectively (Scheme 5.13).[a]



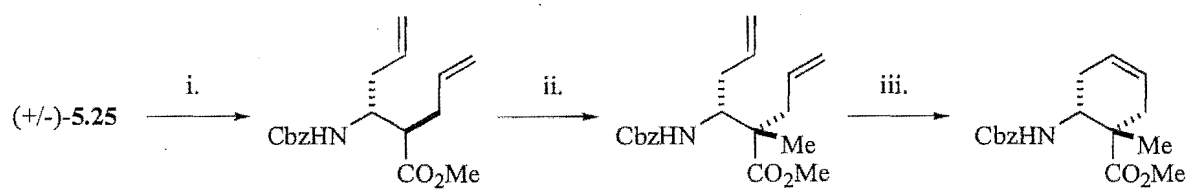
(+/-)-**5.33a** R=Me
(+/-)-**5.33b** R=Et

(+/-)-**5.34a** R=Me
(+/-)-**5.34b** R=Et

Scheme 5.13. *Reagents and Conditions*: **1.40** or **1.42**, benzene, reflux, **5.34a** 96%, **5.34b** 94%

The indicated *trans* stereochemistry is based upon the results of the previous sequence (Schemes 5.6 and 5.9), and literature precedent, details of which were discussed earlier (see Scheme 5.8).[63-66]

With the synthesis of the a-substituted *trans* isomers established, we turned our attention to the preparation of the *cis* isomer **5.36** (Scheme 5.11). This was achieved by reversing the order of the alkylation steps i.e. alkylation of **5.25** with allyl bromide, followed by alkylation with methyl iodide (Scheme 5.14).



(+/-)-**5.26** (+/-)-**5.35** (+/-)-**5.36**

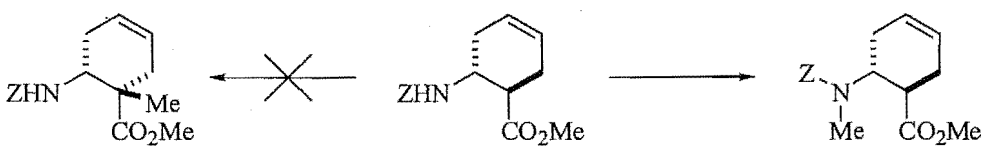
Scheme 5.14. *Reagents and Conditions*: i. LiCl, 2 equiv of LDA, THF, -78° C, allyl bromide, 59%; ii. LiCl, 2 equiv of LDA, THF, -78° C, MeI, 68%; iii. **1.40**, benzene, reflux, 91%.

[a] Comparable results were obtained using Grubbs' second generation catalyst **1.42**.

This resulted in **5.35**, an epimer of **5.33a**, being isolated, as a single diastereoisomer by ^{1}H NMR after purification, in 59% yield. RCM of **5.35**, in refluxing benzene, in the presence of **1.40**, gave the α-substituted *cis* cyclic β-amino acid **5.36**. Purification by silica chromatography resulted in **5.36** being isolated as a single diastereoisomer by ^{1}H NMR, in 91% overall yield.

We have therefore demonstrated that it is possible to prepare either the *cis* or *trans* α-substituted cyclic β-amino acids using this methodology. It is interesting to note that while *cis* isomers of this type have not been used in the preparation of β-peptides, they are of use as inhibitors of matrix metalloproteases.[68]

Having established the synthesis of α-substituted cyclohexenyl β-amino acids, via a double alkylation method, we further investigated the possibility of directly alkylating the α-free aminocyclohexenecarboxylic acid **5.27**, to give the equivalent *cis*-α-substituted derivative **5.36**. To this end, deprotonation of (+/-)-**5.27** was attempted, under standard conditions, in the presence of LDA/LiCl. Methyl iodide was then added and the reaction stirred for 1 h at -78° C, and then allowed to warm to room temperature overnight. Upon workup, ^{1}H NMR analysis of the residue showed that the α-methyl ACHC (+/-)-**5.36** had not been formed. Instead, the presence of a sharp methyl singlet at δ_H 2.84ppm indicated that *N*-methylation had occurred, on the amide nitrogen of (+/-)-**5.27**, to give the *N*-methylated ACHC (+/-)-**5.37** (Scheme 5.15). Purification of the residue, by silica chromatography, gave (+/-)-**5.37** in 78% yield,



(+/-)-**5.36** (+/-)-**5.27** (+/-)-**5.37**

Scheme 5.15. Alkylation of **5.27** with MeI gave the *N*-methylated derivative **5.37**.

It is conceivable that manipulation of the *N*-protecting group i.e. use of phthaloyl instead of carbamate, would eliminate the possibility of *N*-methylation and allow for alkylation to occur. However, further research was not carried out in this area to establish the viability of this method.

5.4 Enantioselective Synthesis of Cyclohexenyl-Based β-Amino Acids

With the basic methodology for the preparation of cyclic β-amino acids in place, we now demonstrated that it was possible to prepare a single enantiomer of the cyclic β-amino acids, given that our strategy had been established using racemic **5.25**. Although numerous methods have been reported for the synthesis of β-amino acids, we chose to make use of Evans' chiral auxiliary (Figure 5.13)[69,70] to prepare an enantiomerically pure sample of **5.25**. This methodology relied upon two key observations: 1) that chiral sodium and lithium imide enolates undergo highly diastereoselective alkylation reactions and 2) hydrolysis, and full recovery, of the chiral auxiliary can be achieved under relatively mild conditions.

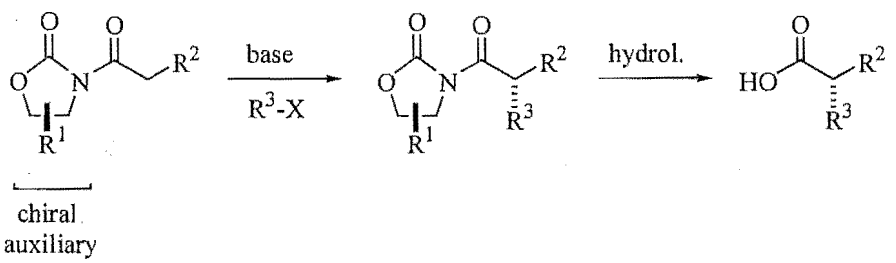
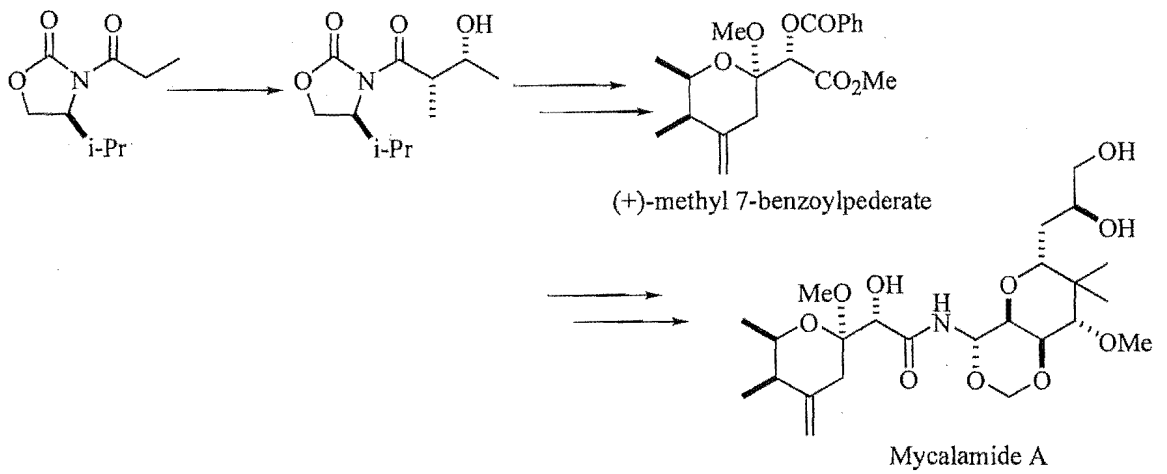


Figure 5.13. Evans' chiral auxiliary methodology for stereoselective alkylations.

The utility of this method lies in its general application towards a variety of acyl imides and the consistently high diastereoselectivities observed during the alkylation reaction. Recently this methodology has received increased attention with it being used extensively in the enantiomeric synthesis of a range of peptidomimetics and natural products. Examples of these

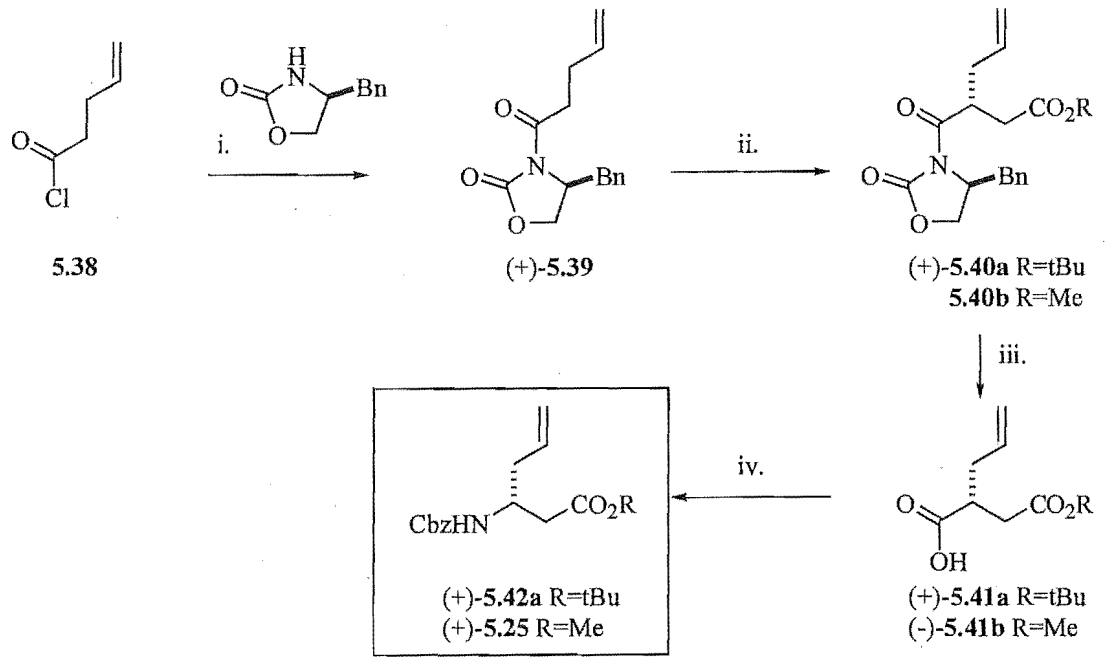
include butyrolactone containing compounds such as (-)-enterolactone,[71] which have been shown to possess protective properties against certain types of cancer; apoptolidin a highly selective and very potent anticancer agent; the mycalamides (see Scheme 5.16),[72-74] compounds isolated from a New Zealand sponge of the genus *Mycale* that exhibit potent antitumor, antiviral and immunosuppressive action via inhibition of T-cell activation; the pectenotoxins (PTXs),[75] a highly selective class of cytotoxic compounds and the agents chiefly responsible for the onset of severe diarrhea and liver damage following the ingestion of bivalves (clams, muscles, scallops etc); as well as a range of carbocyclic nucleosides[76] including carbovir[77] and abacavir (Ziagen),[78,79] compounds that have been shown to be potent inhibitors of HIV replication.



Scheme 5.16. Enantioselective synthesis of Mycalamide A using Evans' chiral auxiliary methodology. Here an asymmetric aldol reaction leads to the desired stereochemistry exhibited by the two adjacent lactone methyl groups.

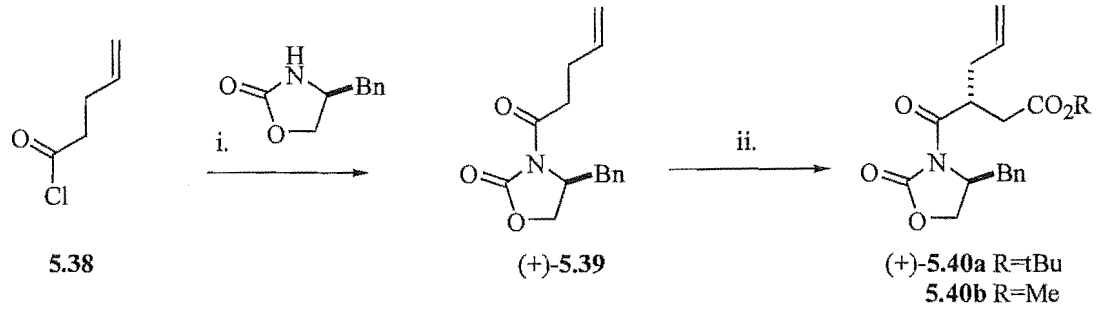
With this methodology seeing diverse application towards the enantioselective synthesis of a wide range of biologically important compounds, including β-amino acids, we felt confident in using this method for the synthesis of **5.25** as a single enantiomer. With an enantiomerically pure sample of **5.25** in hand it was envisaged that its subjection to our α-

allylation, RCM strategy would give the corresponding enantiomerically pure cyclic β-amino acids. The synthesis of (*R*)-**5.25** is outlined in Scheme 5.17.



Scheme 5.17. *Reagents and Conditions*: i. nBuLi, THF, -78° C, 97%; ii. NaHMDS, $BrCH_2CO_2R$, THF, -78° C, **5.40a** 83%, **5.40b** 82%; iii. LiOH-H_2O_2, THF-H_2O, 0° C, **5.41a** 95%, **5.41b** 51%; iv. DPPA, Et_3N, toluene reflux, BnOH **5.42a** 79%, tBuOH **5.25** 71%.

Thus, the chiral reagent (4*S*)-4-Benzyl-1,3-oxazolidin-2-one was deprotonated in the presence of nBuLi, in THF at -78° C. Alkylation, with 4-pentenoyl chloride **5.38**, gave, after purification by silica chromatography, the *N*-acyl oxazolidinone **5.39** in 97% yield (Scheme 5.18). Measurement of the optical rotation for **5.39** gave an $[\alpha]_D$ = +63.4°, c=0.83 $CHCl_3$, a value that corresponded to that reported in the literature (+64.2, c=0.83, $CHCl_3$).[76]



5.38 (+)-**5.39** (+)-**5.40a** R=tBu
5.40b R=Me

Scheme 5.18. *Reagents and Conditions*: i. nBuLi, -78° C, THF, 97%; ii. NaHMDS, $BrCH_2CO_2R$, THF, -78° C, **5.40a** 83%, **5.40b** 82%.

Acyl oxazolidinone (+)-**5.39** was deprotonated, with NaHMDS, and the resulting anion alkylated with *tert*-butyl bromoacetate to give, after recrystallisation, the alkylated imide **5.40a**, as a single diastereoisomer by ^{1}H NMR, in 83% yield. Measurement of the optical rotation for **5.40a** gave an $[\alpha]_D = +51.2$ (c=1.0 CH_2Cl_2).

The asymmetric outcome of this alkylation can be rationalised by considering the enolate intermediate for this reaction. Enolization of chiral imides under these conditions gives rise to a planar, metal chelated (*Z*)-enolate intermediate.[80] This is a result of rotation about the exocyclic amide bond, from the more stable *trans* geometry, to give an intermediate where both carbonyl oxygens are complexed by the metal. The bulky benzyl group on the chiral auxiliary has the effect of blocking the approach of the incoming electrophile from one face of the enolate intermediate.[69] This leads to exceptionally high diastereoselectivities being obtained during alkylation. Figure 5.14 illustrates this concept where θ represents the angle of approach of the incoming electrophile.

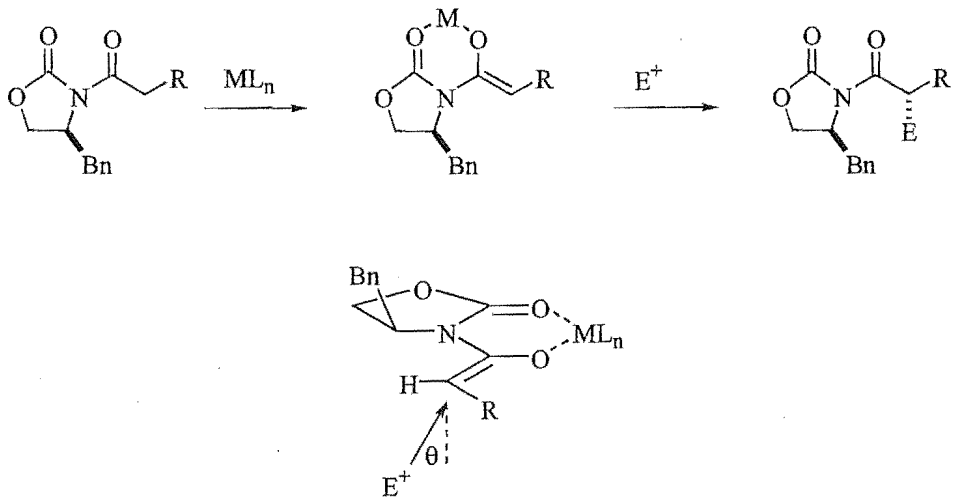


Figure 5.14.

It has been observed that the imide alkylation diastereoselectivities exhibit a modest increase with the increasing size of the alkyl groups adjacent to the enolate carbon. A suggested explanation for this is that the approaching electrophile must alter its path, due to steric interactions with a large R group, thereby amplifying the influence of the benzyl group on the chiral auxiliary, and resulting in increased diastereoselectivities.[69] This selectivity was observed during the synthesis of **5.40a** with the added strength of this method being in the ease of separation of the isomers by recrystallisation.

The relative configuration of **5.40a** was established via x-ray crystallographic analysis, with **5.40a** crystallising in the space group $P2_12_12_1$ with 4 superimposable molecules in the unit cell. A perspective drawing of the solid-state structure of **5.40a**, with atom labelling, is shown in Figure 5.15.

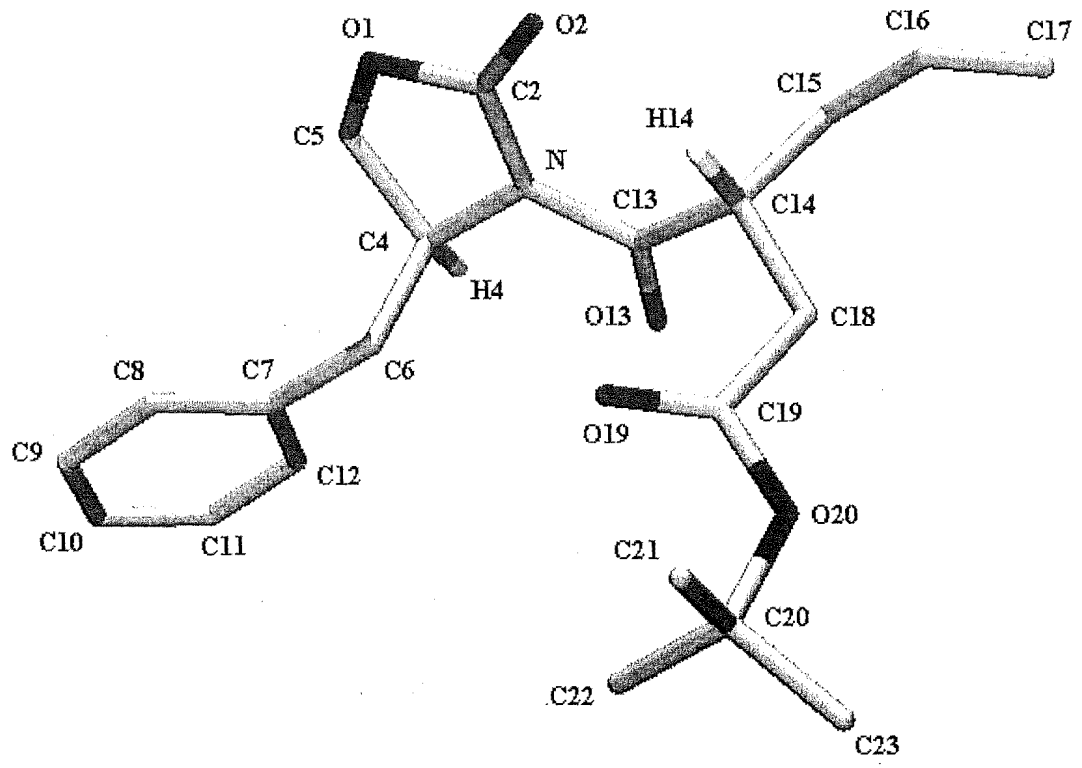
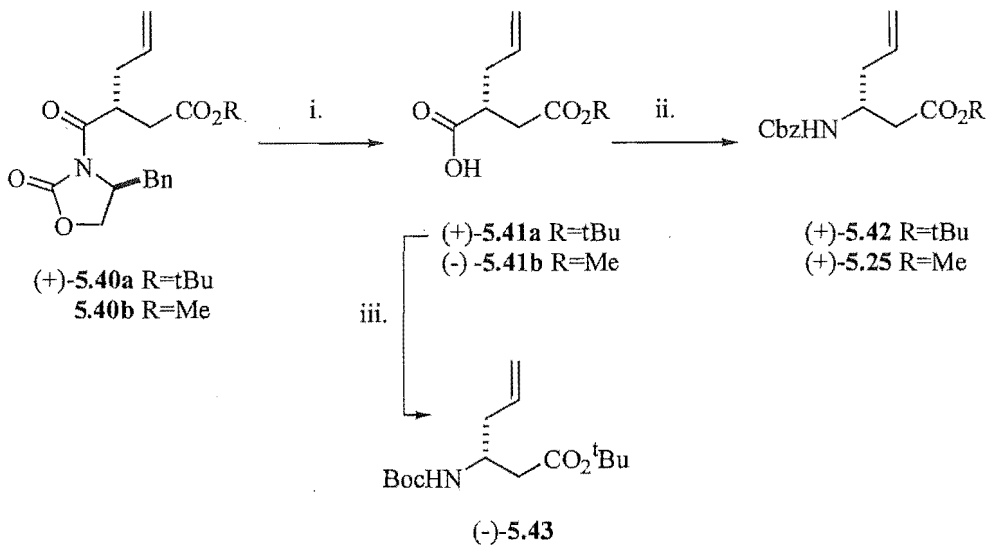


Figure 5.15. Perspective drawing of **5.40a** with atom labelling

The absolute stereochemistry at C4 of **5.40a**, determined by the use of (4*S*)-4-benzyl-1,3-oxazolidin-2-one as the starting chiral auxiliary, allowed the absolute stereochemistry at C14 to be assigned as *R*. Note that the amide-like 'backbone', represented by C4-N-C13-C14, is shown to adopt the more stable *trans* geometry about the N-C13 bond. This contrasts the *cis*-configuration adopted by the metal-chelated enolate intermediate, prior to alkylation (Figure 5.14).

Alkylation of **5.39**, with methyl bromoacetate was also carried out in a similar manner to that described for the preparation of (+)-**5.40a** (Scheme 5.18). The resulting imide **5.40b** was isolated, after purification by silica chromatography, as a single diastereoisomer by ^{1}H NMR, in 82% yield.

Next, cleavage of the chiral auxiliary of (+)-**5.40a** was carried out using lithium hydroperoxide. Addition of lithium hydroxide and hydrogen peroxide to a solution of (+)-**5.40a**, in THF, at 0° C, gave the differentially protected diacid **5.41a** in 95% yield. The chiral auxiliary was also recovered from this reaction in 98% yield.



Scheme 5.19. *Reagents and Conditions*: i. LiOH-H_2O_2, THF-H_2O, 0° C, **5.41a** 95%, **5.42b** 51%; ii. DPPA, Et_3N, toluene reflux, BnOH, **5.42** 79%, **5.25** 71%; iii. DPPA, Et_3N, toluene reflux, ^tBuOH, **5.43** 68%.

A Curtius rearrangement procedure was then utilised for the conversion of **5.41a** to the optically active Cbz-protected β-allyl glycine *tert*-butyl ester **5.42**.[81] Thus, treatment of **5.41a** with diphenylphosphoryl azide and triethylamine,[69] at room temperature for 30 minutes, followed by gentle heating to reflux, resulted in the formation of an intermediate isocyanate. Subsequent trapping of the isocyanate, with benzyl alcohol, gave after purification by radial chromatography, **5.42** in 79% yield. Measurement of the optical rotation for **5.42** gave an $[\alpha]_D = +1.8°$ (c=1.0, CH_2Cl_2) signifying the compound to be optically active. The mechanism for this reaction is shown in Figure 5.16.

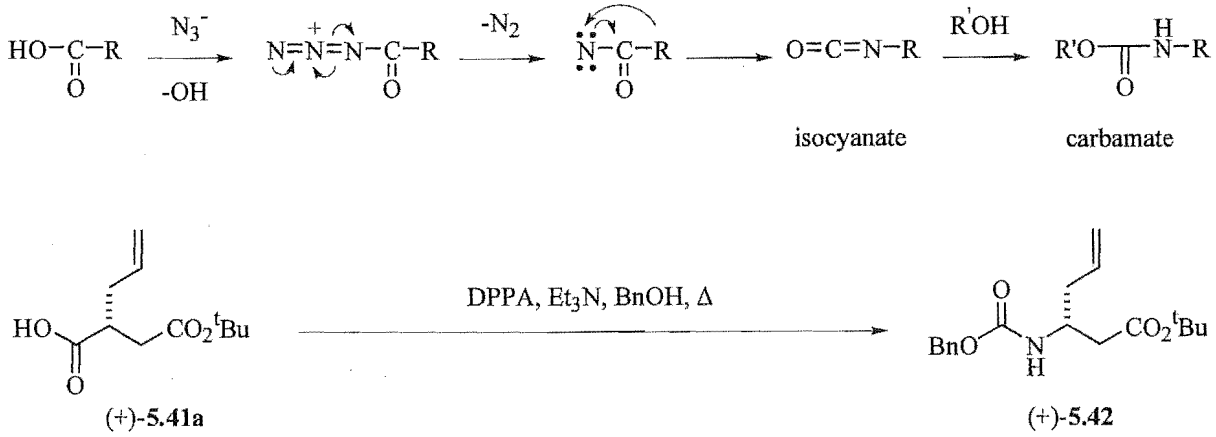


Figure 5.16. Mechanism of the Curtius rearrangement, used in the conversion of (+)-**5.41a** to (+)-**5.42**.

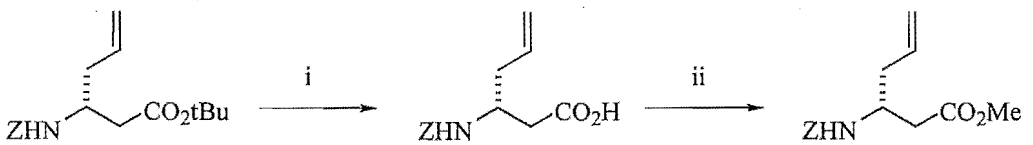
Here, pyrolysis of the acyl azide, followed by a rearrangement, results in a stable, but seldom isolated, isocyanate intermediate. Hydrolysis of the isocyanate in the presence of water, or alcohol, yields the corresponding amine or carbamate.

Diphenylphosphoryl azide was used to generate the azide from (+)-**5.41a**. The azide rearranges to give an isocyanate which is trapped by benzyl alcohol to give carbamate (+)-**5.42**. As an alternate method for the preparation of (+)-**5.42** using a two-step procedure reported by Yakushijin *et al*, was also carried out whereby the mixed anhydride of (+)-**5.41a** was generated, and treated with sodium azide in the presence of triethylamine. Hydrolysis of the isocyanate, with benzyl alcohol, yielded (+)-**5.42** in comparable yield to the procedure utilizing DPPA. However, despite comparable yields being obtained on a small scale, in our hands this latter method was not amenable to application on a large scale. Therefore DPPA was used as the reagent of choice for the generation of the isocyanate in this reaction.

Curtius rearrangement was also carried out on (+)-**5.41a** using *tert*-butyl alcohol as the trapping agent, to give the known Boc-protected derivative **5.43**, as a single isomer by ^1H NMR, in 68% yield (Scheme 5.19). Measurement of the optical rotation for **5.43** gave an $[\alpha]_D$

= -9.8° (c=1.1 MeOH), a value that closely agreed with that reported in the literature (-10.09°, c=1.1 MeOH).[70] This was an important result as the agreement in optical rotation with this known compound established the enantioselective integrity of the synthesis. For the preparation of (-)-**5.43**, the moderate yield of the reaction can be attributed to the tendency of the more sterically hindered tertiary alcohol to give lower yields during hydrolysis of the isocyanate.

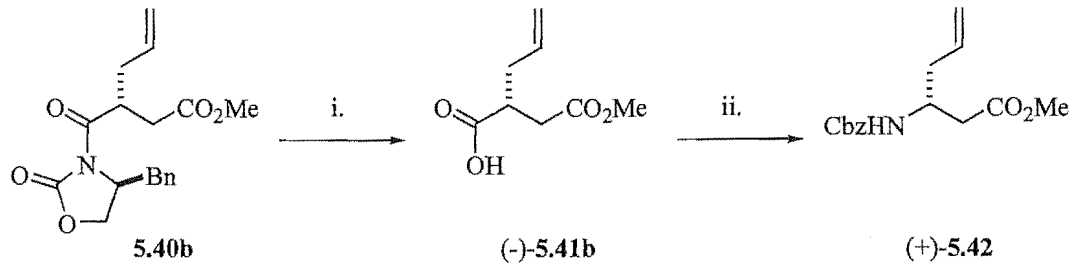
Next, (+)-**5.42** was converted to **5.25** such that the sequence outlined in Scheme 5.6 could be repeated using optically active material. This was achieved by hydrolysis of the *tert*-butyl ester of (+)-**5.42**, with trifluoroacetic in dichloromethane, to give the free acid **5.44**, which was re-esterified with diazomethane, to form the Cbz-protected β-allyl glycine methyl ester **5.25**. Measurement of the optical rotation for **5.25** gave an $[\alpha]_D$ = +4.2° (c=2.0 $CHCl_3$), a value in close agreement with that reported in the literature (+4.7°, c=2.0 $CHCl_3$).[82]



(+)-**5.42** **5.44** (+)-**5.25**

Scheme 5.20. *Reagents and Conditions*: i. TFA, CH_2Cl_2, Me_2S, 0° C to rt, 90%; ii. CH_2N_2, Et_2O, 0° C, 100%.

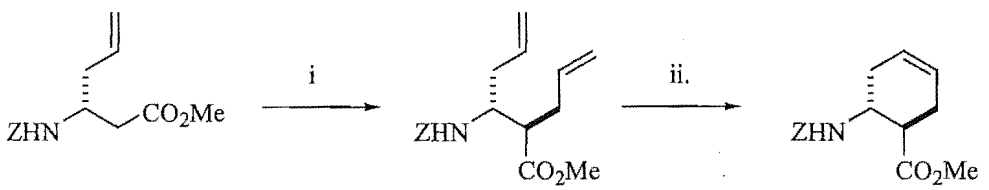
Methyl ester (+)-**5.25** was also prepared from (+)-**5.39**, by alkylation with methyl bromoacetate (Schemes 5.18 and 5.19), to give **5.40b**, as a single diastereoisomer by ^1H NMR, in 82% yield. Hydrolysis of the chiral auxiliary from this compound gave (-)-**5.41b**, in a moderate yield of 51%, which was subjected to Curtius rearrangement conditions, quenching with benzyl alcohol, to give (+)-**5.25** directly in 71% yield (Scheme 5.21).



5.40b (-)-**5.41b** (+)-**5.42**

Scheme 5.21. *Reagents and Conditions*: i. $LiOH\text{-}H_2O_2$, $THF\text{-}H_2O$, 0° C, 51%; ii. DPPA, Et_3N, toluene reflux, BnOH, 71%.

Finally, synthesis of the optically active cyclic β-amino acid was carried out as described for the racemic series (see Scheme 5.6). In particular, (+)-**5.25** was allylated with allyl bromide, via a doubly lithiated intermediate, to give the diallylated derivative (+)-**5.26**, as a single diastereoisomer by ^1H NMR, in 49% yield (Scheme 5.22). Measurement of the optical rotation for (+)-**5.26** gave an $[\alpha]_D = +8.2°$, (c=1.0 CH_2Cl_2).



(+)-**5.25** (+)-**5.26** (-)-**5.27**

Scheme 5.22. *Reagents and Conditions*: 2LDA, LiCl, allyl bromide, THF, -78° C, 49%; ii. **1.40** or **1.42**, benzene reflux, 91%.

Diene (+)-**5.26** was then subjected to ring-closing metathesis conditions to give, after purification by silica chromatography, (-)-**5.27** in 91% yield, as a single diastereoisomer by ^1H NMR. Measurement of the optical rotation for (-)-**5.27** gave an $[\alpha]_D = -31.2°$, (c=1.0, $CHCl_3$), a value in close agreement with that reported in the literature (-33.2°, c=1.0 $CHCl_3$).[83]

To further confirm the validity of this methodology for the synthesis of ACHC's, (-)-**5.27** was hydrogenated, and reprotected with benzylchloroformate, in the presence of DIEA and

DMAP, to give, after purification by silica chromatography, saturated (-)-**5.28**, in 75% yield (Scheme 5.23).

(-)-**5.27** (-)-**5.28**

Scheme 5.23. *Reagents and Conditions*: H_2, Pd-C, MeOH then DIEA, $PhCH_2OCOCl$, DMAP, 75%.

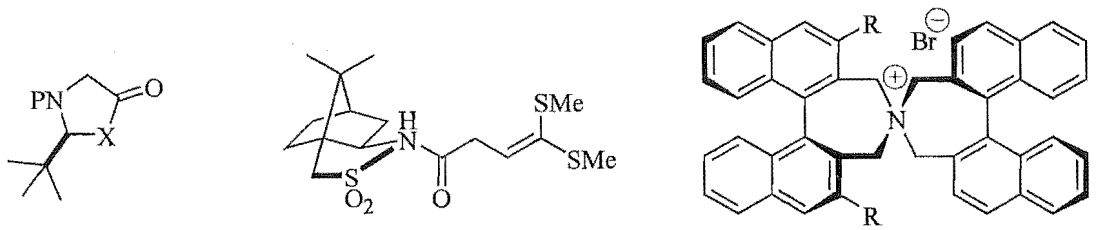
Measurement of the optical rotation of (-)-**5.28** gave an $[\alpha]_D = -18.4°$ (c=0.9, $CHCl_3$), a value that is in agreement with that reported in the literature (-18°, c=0.9 $CHCl_3$).[59]

5.5 Enantioselective Synthesis of Allyl Glycine

An alternate strategy for the enantioselective synthesis of cyclic β-amino acids of type **5.53** (see introduction) involves a different optically active precursor to that used in Section 5.4. For preparations of cyclic β-amino acids **5.27** (Scheme 5.6), **5.34** and **5.36** (Scheme 5.11), racemic allyl glycine was employed in the preparation of the key precursor **5.25**. (see Scheme 5.7). Optically active (+)-**5.25** was subsequently prepared through the used of Evans' chiral auxiliary chemistry (Scheme 5.17). It was envisaged that the use of optically active allyl glycine as a precursor to **5.25** would also result in an efficient synthesis of enantiomerically pure compounds of this type. Hence, efforts were directed towards the enantioselective synthesis of allyl glycine.

The importance of naturally occurring proteinogenic amino acids is well known, with the preparation of this class of compound, usually by microbiological means,[84] well established in industry. However, the search for cheap and convenient methods for the synthesis of non-

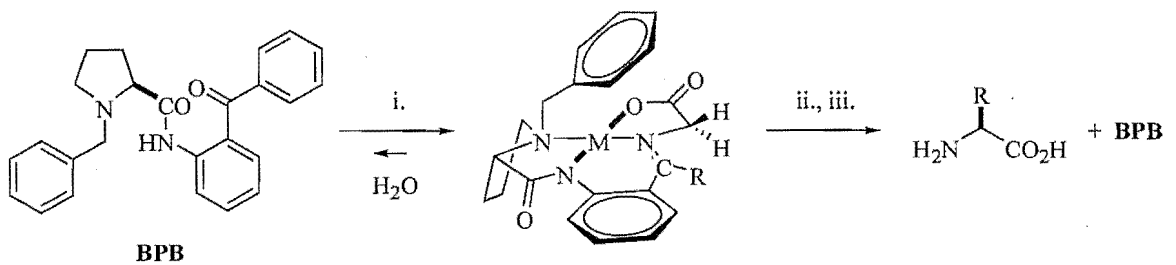
natural, or isotopically labelled amino acids still goes on. At present, there are several excellent general methods for the synthesis of non-natural amino acids,[85] with the most important commercial examples being Seebach's and Oppolzer's derivatives **5.45**[86] and **5.46**[87] respectively, and O'Donnell's stereospecific alkylation of glycine-derived benzimidines[88], for which an efficient chiral phase-transfer catalyst **5.47** has recently been developed (Figure 5.17).[89]



5.45 X=O, NMe **5.46** **5.47** R=β-Naph, 3,4,5-F_3-Ph

Figure 5.17. Agents for the enantioselective synthesis of both natural and non-natural amino acids

Another method, developed by Belokon *et al*, makes use of Ni(II) complexes of Schiff bases of (S)-2-[N-(N'-benzylprolyl)-amino]benzophenone (BPB) and glycine, to achieve high asymmetric induction for the synthesis of α-amino acids (Scheme 5.24).

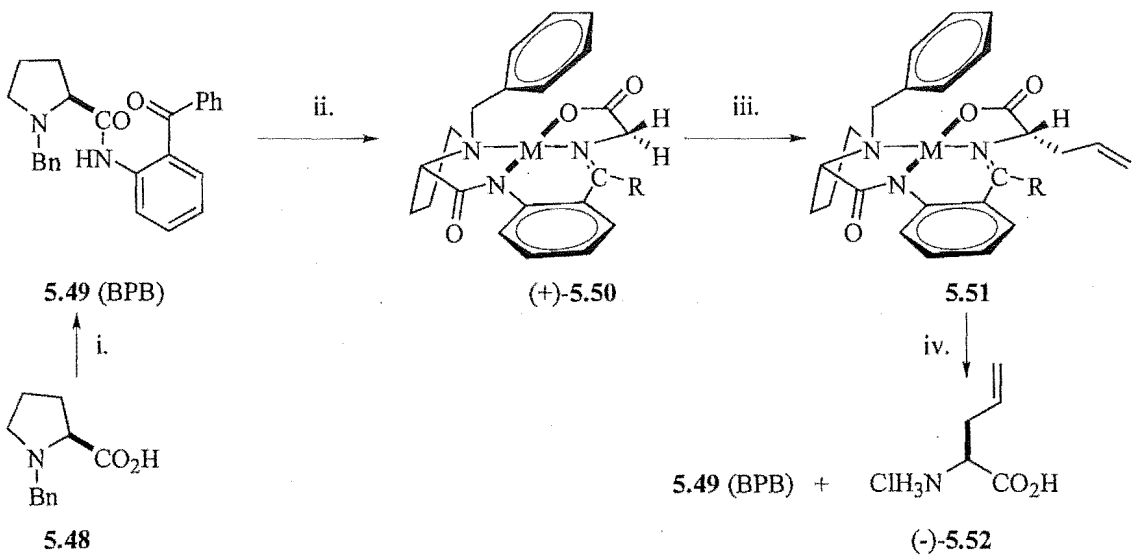


Scheme 5.24. *Reagents and Conditions*: i. $Ni(NO_3)_2.6H_2O$, glycine, MeOH, 50° C then MeONa; ii. NaOH, RX, CH_3CN, rt; iii.. MeOH, 2N HCl, reflux, ion-exchange Dowex 50X8 H^+.

Originally developed as an artificial analogue of pyridoxal 5'-phosphate (PLP)-dependent enzymes,[90] this system offers several advantages over other existing methods. These include the simplicity of operation, ambient temperatures of the reaction media, very high

concentrations of reagents, the use of cheap bases (NaOH, KOH, MeONa, NaH), and the convenient and facile recovery of the amino acids and the chiral auxiliary. The simple nature of this methodology and the use of cost-effective and recoverable reagents, makes this method particularly attractive in an environmental context.[91]

Therefore, the preparation of (*S*)-allyl glycine hydrochloride **5.52** was carried out using the method reported by Belokon *et al* (Scheme 5.25).[92]



Scheme 5.25. *Reagents and Conditions*: i. $SOCl_2$, CH_2Cl_2, -30° C then 2-aminobenzophenone, 58%; ii. $Ni(NO_3)_2.6H_2O$, glycine, MeOH, 50° C then NaOMe, 90%; iii. NaOH, allyl bromide, CH_3CN, rt, 94%; iv. MeOH, 2N HCl, reflux, ion-exchange Dowex 50X8 H^+, 87%.

Thus, thionyl chloride was added to benzyl-(*S*)-proline **5.48**, in CH_2Cl_2 at -30° C, followed by 2-aminobenzophenone, and the solution stirred at -30° C for 10h. Workup, followed by extraction and recrystallisation, gave BPB **5.49** in 58% yield. Subsequent availability of BPB from commercial sources negated this step in future preparations. Analysis of the ^1H NMR spectrum, and melting point, of **5.49** prepared by the method described in Scheme 5.25, gave data consistent with that observed for the commercial sample.

Next, a suspension of BPB **5.49**,[f] $Ni(NO_3)_2.6H_2O$, and glycine was warmed to 50° C under argon. A solution of 1.2N MeONa was quickly added, inducing a colour change from pale green to dark red/orange, and the mixture was stirred rigorously for 2h at 50° C. Addition of water, followed by extraction with $CHCl_3$, gave a red oil that, after purification by silica chromatography, gave **5.50**, as a red solid in 90% overall yield. Measurement of the optical rotation of **5.50** gave an $[\alpha]_D$ = +1880° (c=0.125, MeOH),[g] a value that approximated that reported in the literature (+2006, c=1.0, MeOH).[93]

With the key Ni(II)-BPB-glycine complex (+)-**5.50**.in hand, we proceeded with its alkylation using allyl bromide. To a stirred mixture of (+)-**5.50**, in dry CH_3CN, was added finely powdered NaOH, followed by allyl bromide, and the reaction stirred at rt for 3h. Treatment of the solution with 0.1M HCl, followed by extraction with CH_2Cl_2 gave, after purification by silica chromatography, **5.51** as a single isomer by ^1H NMR, in 94% yield. Subsequent hydrolysis of the complex was carried out by refluxing a solution of **5.51** in MeOH/2N HCl, followed by the addition of concentrated ammonia at room temperature. BPB **5.49**, was recovered in 97% yield by extraction with CH_2Cl_2. The aqueous layer was concentrated and chromatographed with a cation exchange resin (Dowex 50X8 H^+) to give allyl glycine **5.52**, as a hydrochloride salt, in 82% yield. Measurement of the optical rotation for **5.52** gave an $[\alpha]_D$ = -6.2° (c=2.1, 6N HCl), a value in close agreement with that reported in the literature (-6.4°, c=2.1, 6N HCl).[94]

It is envisaged that the use of (-)-**5.52** in the preparation of optically active **5.25** will make the stereoselective alkylation sequence described in Schemes 5.6 and 5.11 a more efficient method for the synthesis of enentiomerically pure aminocyclohexenylcarboxylic acids such as **5.27**, **5.34** and **5.36**.

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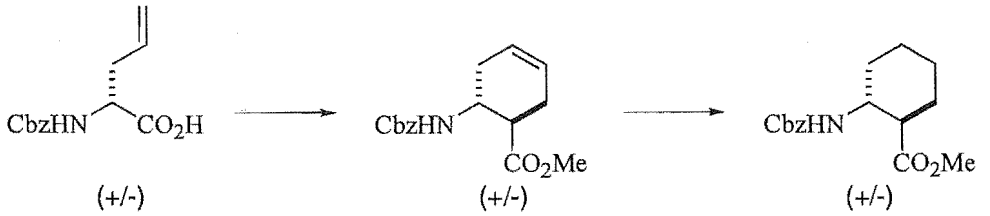
[f] A commercial sample of BPB was utilized for this reaction.

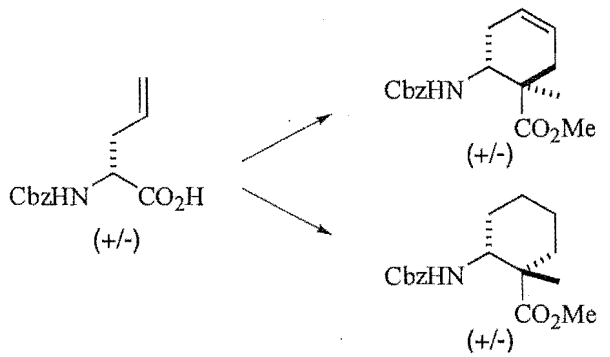
[g] Problems were encountered during the measurement of the optical rotation of **5.50** due to its large [α] value. Large dilutions were needed to obtained values suitable for comparison with those reported in the literature.

5.6 Conclusion and Future Work

In summary, we have demonstrated a new and simple procedure for the synthesis of cyclohexenyl β-amino acids based on RCM chemistry. This methodology was used to prepare the unsubstituted *trans* cyclic β-amino acids **5.27** and **5.28** from the simple diene precursor **5.26**.

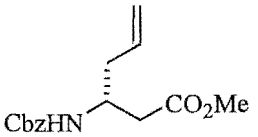


We have also demonstrated that an α-substituent, as in **5.34** and **5.36**, can be introduced stereoselectively, by employing an allylation/alkylation sequence, the order of which defines the absolute stereochemistry of the product. This second class of cyclic β-amino acids represents a new and important addition to the family of compounds.



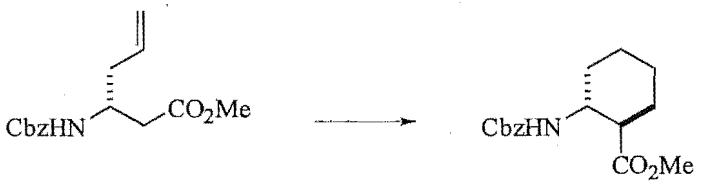
Finally, we have shown that optically active (-)-**5.27** and (-)-**5.28** can be prepared using optically active (+)-**5.25**. This was obtained through the use of Evans' chiral auxiliary chemistry to carry out a stereoselective alkylation of a chiral imide **5.39**, with *tert*-butyl bromoacetate, to give the key alkylated imide (+)-**5.40a**, as a single diastereoisomer by ^{1}H NMR. The solid-state structure of (+)-**5.40a**, and its associated space group ($P2_12_12_1$), were subsequently determined by x-ray crystallography, allowing the absolute stereochemistry of (+)-**5.40a** to be assigned as (*S*,*R*). Hydrolysis of the chiral auxiliary from (+)-**5.40** gave the differentially protected diacid **5.41a** that was converted, via a Curtius rearrangement, to the

Cbz-protected β-amino acid tert-butyl ester 5.42. Hydrolysis of **5.42**, followed by re-esterification with diazomethane gave optically active (+)-**5.25** is good yield. Diacid **5.41a** was also converted to the known Boc-protected β-amino acid (-)-**5.43**, the optical rotation of which closely agreed with the literature value.



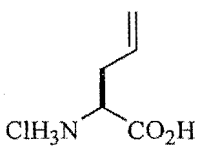
(+)-**5.25**

Compound (+)-**5.25** was also prepared by alkylation of the chiral imide **5.39**, with methyl bromoacetate, to give the alkylated imide **5.41b**. Hydrolysis of the chiral auxiliary from **5.41b** followed by a Curtius rearrangement, in the presence of benzyl alcohol, gave (+)-**5.25** directly in moderate yield. Use of (+)-**5.25** in the preparation of optically active (-)-**5.27** and (-)-**5.28** provided compounds with optical rotations that corresponded to those reported in the literature.



(+)-**5.25** (-)-**5.28**

An alternate source of optically active **5.25** was also explored with the preparation of optically active allyl glycine.HCl (-)-**5.52** via the stereospecific alkylation of the Ni(II)-BPB-glycine complex **5.50**, with allyl bromide. Measurement of the optical rotation of **5.52** gave a value in close agreement with that reported in the literature.



(-)-**5.52**

Future work in this area lies in the incorporation of cyclohexyl-based β-amino acid derivatives into novel β-peptides to explore new and interesting forms of secondary structure.

5.7 References for Chapter Five

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CHAPTER SIX

SYNTHESIS OF α-SUBSTITUTED CYCLOPENTENYL-BASED β-AMINO ACIDS BY RING–CLOSING METATHESIS

6.1 Introduction

2-Aminocyclopentanecarboxylic acids (ACPCs), and derivatives thereof, have shown a variety of interesting biological activities. A decade ago, (1*R*,2*S*)-ACPC, an antifungal antibiotic also known as cispentacin (Figure 6.1), was isolated independently by two Japanese groups from *Basicillus cereus*,[1,2] and *Streptomyces setonii*.[3,4]

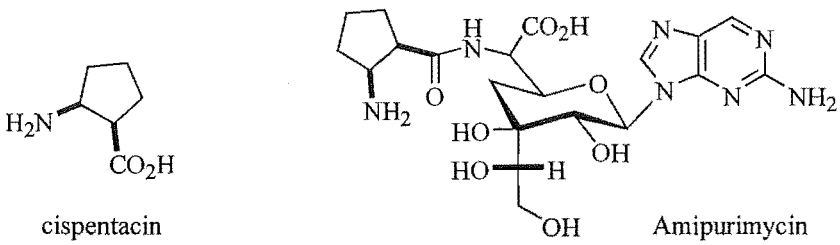


Figure 6.1. 2-Aminocyclopentane carboxylic acids, and their derivatives show a variety of biological activities.

cis-2-ACPC was also found to be a component of amipurimycin, an antibiotic isolated from *Streptomyces novoguineensis* that is strongly active, both *in vitro* and *in vivo*, against *Pyricularia oryzae*, the organism responsible for rice blast disease. The *L*-aspartyl dipeptides of all four stereoisomers of 2-ACPC have been used to probe a molecular model of taste (Figure 6.2).[5] Here, Asp-*trans*-(1*R*,2*R*)-2-ACPC methyl ester and Asp-*cis*-(1*S*,2*S*)-2-APAC methyl ester were found to assume an *L*-shaped conformation required for sweetness, with Asp-*trans*-(1*S*,2*S*)-2-ACPC methyl ester tasting bitter, and Asp-*cis*-(1*R*,2*S*)-APAC methyl ester proving tasteless, as it fitted neither of the models for bitter or sweet.

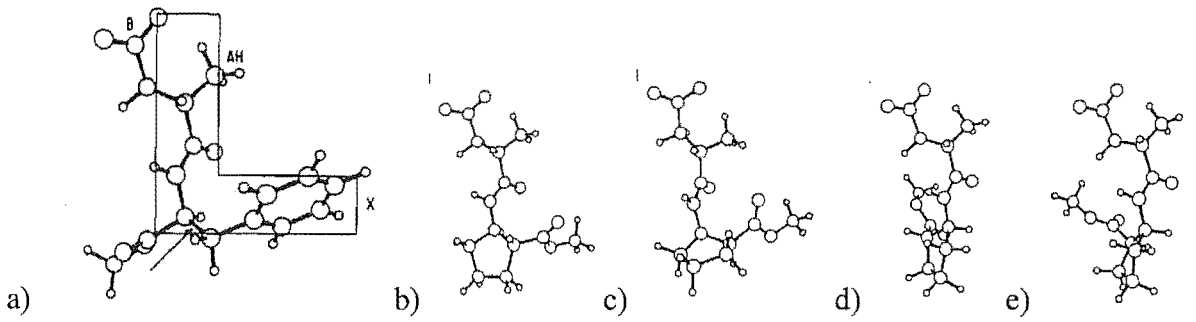


Figure 6.2. a) Model of the sweet taste with *L*-Asp-*L*-PheOMe superimposed, b) Asp-*trans*-(1*R*,2*R*)-2-ACPC.OMe, c) Aps-*cis*-(1*S*,2*R*)-2-ACPC.OMe, d) Asp-*trans*-(1*S*,2*S*)-2-ACPC.OMe, e) Aps-*cis*-(1*R*,2*S*)-2-ACPC.OMe.

Recently, increasing work has been devoted to the study of homogenous, sequence-specific oligomers that mimic various aspects of the folding and organization of polypeptides. Groups led by Seebach and Gellman have independently shown that polyamide sequences composed of C^2- and/or C^3-substituted β-amino acids, adopt stable helical structures, both in solution and the solid-state (Figure 6.3).

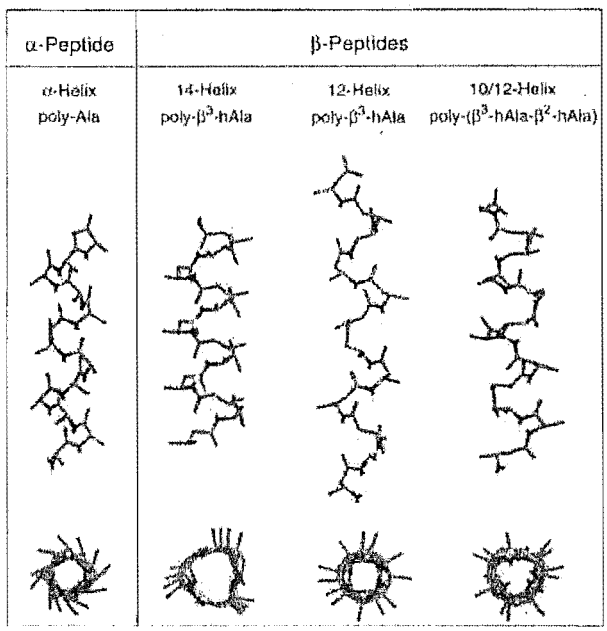


Figure 6.3. Structure of the α-helix compared with the helical structures adopted by β-peptides, namely, the 14-helix, 12-helix, and 10/12-helix.[6]

Gellman further demonstrated that β-peptides containing the conformationally strained cyclic amino acids *trans*-2-aminocyclohexanecarboxylic acid (ACHC), or *trans*-2-aminopentanecarboxylic acid (ACPC), adopted stable helical conformations much more readily than their acyclic counterparts. Systematic studies of ACHC-containing oligomers versus ACPC-containing oligomers, has revealed inherent preferences for different conformations. The cyclohexyl ring of ACHC stabilises the θ torsion angle to a value near ±60°, which specifically stabilises the 14-helical conformation (Figure 6.4, see also Chapter 5). The smaller ring size of ACPC tends θ towards larger values, thereby favouring a novel helical form, the 12-helix.

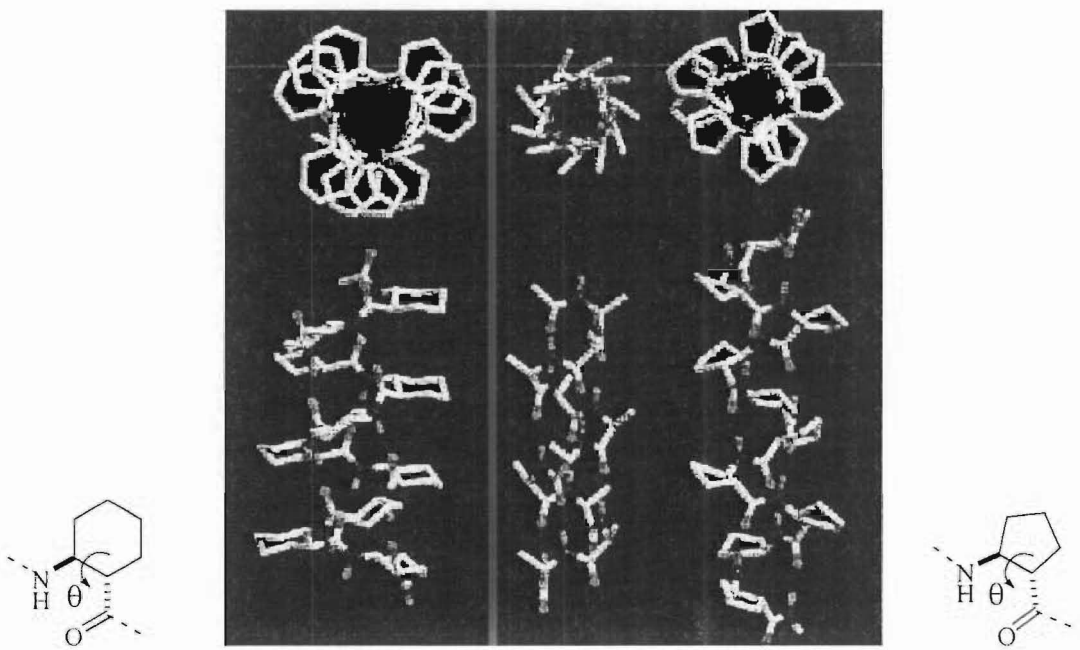
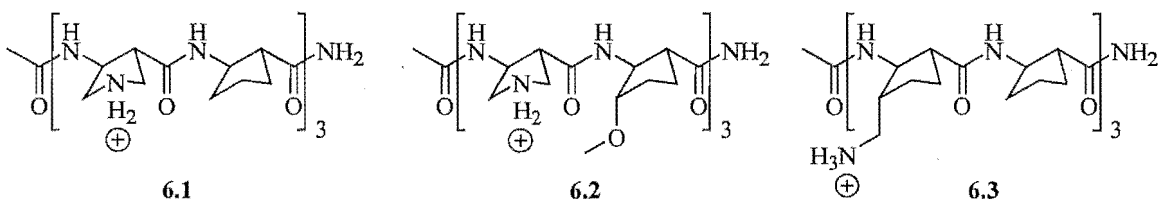


Figure 6.4. Helical conformations adopted by β-peptides composed of *trans*-ACHC (left), and *trans*-ACPC (right), compared with that of the α-helix (centre).

The structure of the 12-helix is stabilised by a series of hydrogen bonds between the amide carbonyl groups at position *i*, and the amide proton at position *i*+3, with the helix repeating approximately every 2.5 residues. The 12-helix also shows the same polarity as the α-helix, with the amide protons exposed from the *N*-terminal end of the helix. This is in contrast to the

14-helix adopted by ACHC-containing β-peptides, which show an opposite polarity to that of the α-helix. This ability to switch between two completely different β-peptide helices, by means of a relatively small modification in residue structure, calls attention to a significant difference between α-amino acid and β-amino acids as building blocks. Greater control can therefore be maintained over the intrinsic secondary structure propensity of β-amino acid residues than is possible with α-amino acids residues. This has been illustrated in the preparation of a number of water-soluble derivatives. Oligomers of ACPC, while forming extremely stable helical structures in organic solvents, are not soluble in water. To address this limitation, derivatives incorporating such residues as *trans*-aminopyrrolidine-4-carboxylic acid (APC) (**6.1** and **6.2**), and 3-substituted ACPCs (**6.2** and **6.3**), have been prepared and found to adopt stable helical conformations in water.[7]



6.1 **6.2** **6.3**

Figure 6.5. β-Peptides incorporating derivatised ACPCs are water-soluble.

Compounds of this type are of interest for their potential to mimic the α-helical conformations adopted by docking substrates in a number of receptor-mediated processes. Seebach *et al* have recently reported examples of helical β-peptides that exhibit a variety of biological properties ranging from the inhibition of fat and cholesterol absorption, to potent antimicrobial activity. DeGrado *et al* subsequently reported derivatives that possessed highly potent cell-lytic activity,[8] with compounds of this type designed to kill bacteria by disrupting the cellular membranes of their targets (Figure 6.6). The activity of these compounds has been directly related to their ability to adopt a helical conformation in solution.

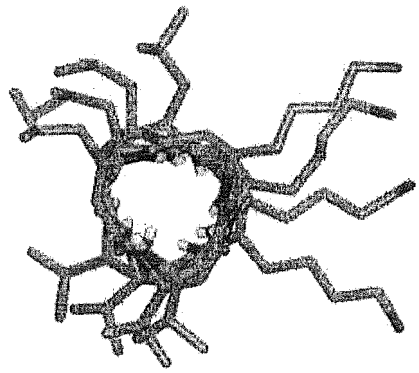
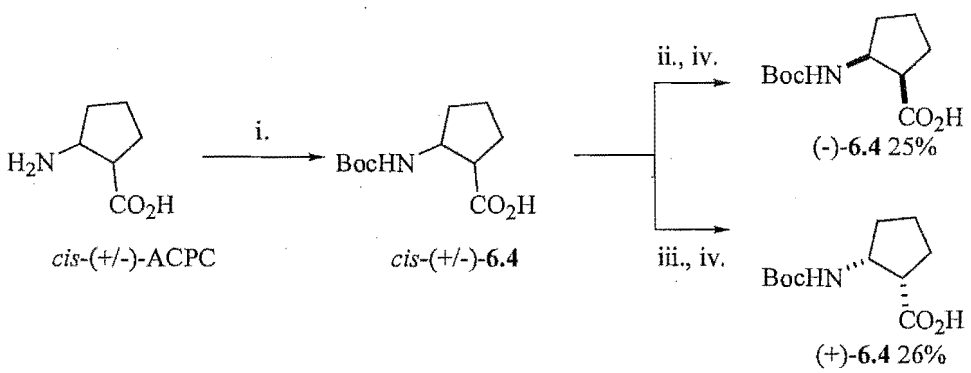


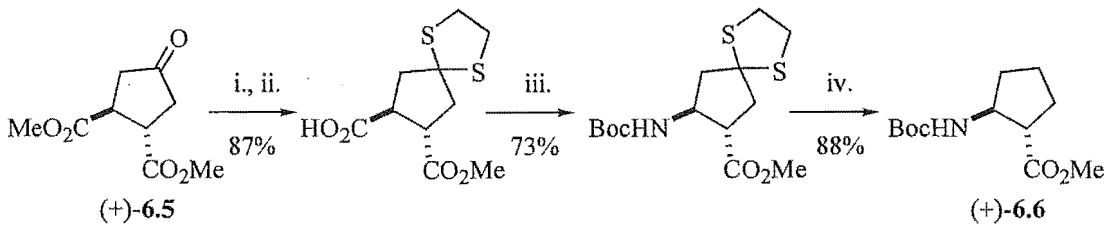
Figure 6.6. Helical β-peptides have been shown to exhibit potent antimicrobial activity.[6]

On this basis, the synthesis of aminocyclopentanecarboxylic acids has become the subject of several recent investigations that has included traditional resolution, enzymatic resolution, and asymmetric synthesis. It has been known for some time that the selective reduction of anthranilic acid over Adams catalyst in acetic acid, or over a Rh-Al catalyst, gives racemic *cis*-ACPC as the main product.[9-12] However, resolution of racemic *cis*-ACPC was only reported after the isolation of natural cispentacin, with crystallisation of **6.4**, in the presence of ephedrine, allowing separation of the diastereoisomers for use in synthesis (Scheme 6.1).[5]



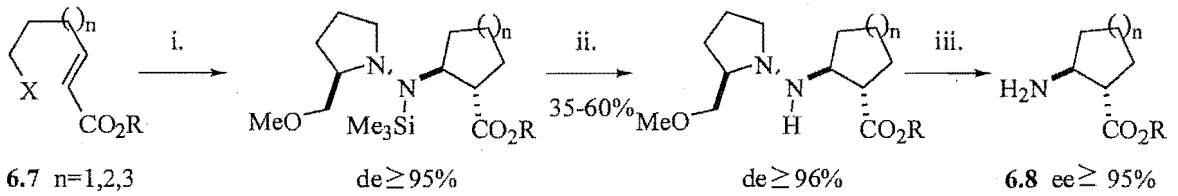
Scheme 6.1. *Reagents and Conditions*: i.Boc_2O, $HaHCO_3$, THF/H_2O (3:1), rt; ii. (+)-ephedrine, EtOAc, crystallisation; iii. (-)-ephedrine, EtOAc, crystallisation; iv. $NaHSO_4$

Notesberg *et al* devised a simple four step synthesis of both enantiomers of *trans*-2-ACPC,[13] with the key starting components, either enantiomer of **6.5**, readily available in high enantiomeric purity by enzymatic resolution.[14] Hydrolysis and Curtius rearrangement, followed by removal of the thioacetal, gave *trans*-2-ACPC enantiomers in good yield.



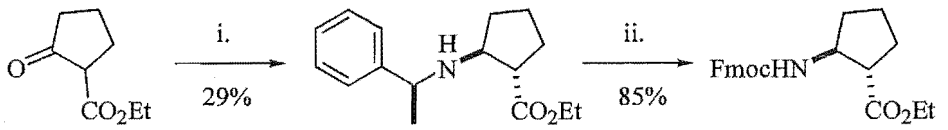
Scheme 6.2. *Reagents and Conditions*: i. $HSCH_2CH_2SH$, $SnCl_4$, CH_2Cl_2, rt; ii. NaOH, H_2O/dioxane (1:1), rt; iii. DPPA, Et_3N, *tert*-butanol, 80° C; iv. Raney-Ni, MeOH, reflux.

Homologues of (1*S*,2*S*)-*trans*-ACACs have been prepared in a relatively short pathway by Enders *et al.* Addition of the chiral ammonia equivalent lithiated (*S*)-(-)-2-methoxymethyl-1-trimethylsilylaminopyrrolidine (TMS-SAMP) to ω-halo-substituted enoate **6.7**, followed by reduction, gave an *N*-silyl intermediate with 96-98% diastereoselectivity (Scheme 6.3).[15-17] Desilylation, reductive *N-N* bond cleavage, followed by hydrolysis and ion-exchange chromatography gave **6.8** in high ee.



Scheme 6.3. *Reagents and Conditions*: i. a) TMS-SAMP, n-BuLi, THF, -78° C, b) HMPA, -78° C, (n=2,3), c) $NaHCO_3$, H_2O; ii. SiO_2, EtOAc, (HCl); iii. a) Raney-Ni/H_2, MeOH, b) 6N HCl, c) Dowex 50WX8-200.

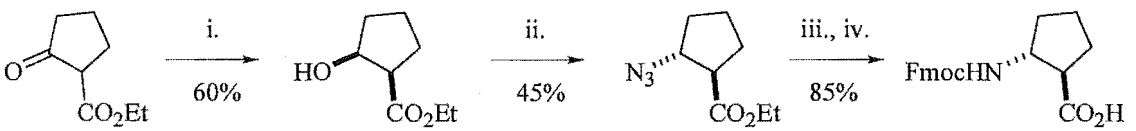
Gellman used α-methylbenzylamine in the reductive amination of **6.9**, to obtain either isomer of **6.10**, for use in the preparation of helical foldamers (Scheme 6.4.).[18]



6.9 6.10

Scheme 6.4. *Reagents and Conditions*: i. a) (*S*)-(-)-α-methylbenzylamine, AcOH, b) $NaBH_3CN$, c) HCl, d) recrystallisation; ii. a) LiOH, H_2O, b) H_2, 10% Pd/C, c) Fmoc-Osu.

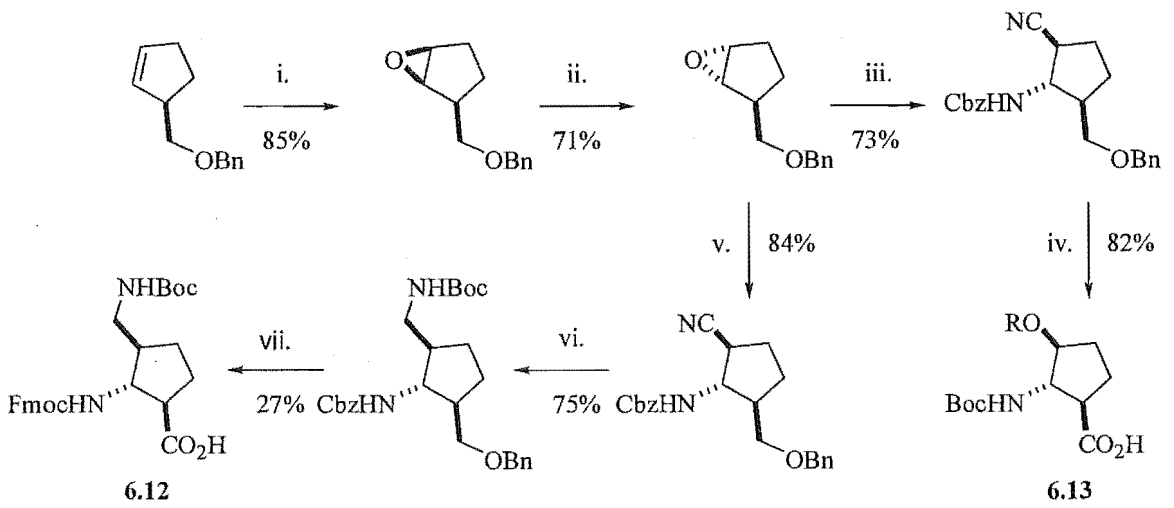
Alternatively, enzymatic methods, using Baker's yeast, were employed to obtain (1*R*,2*R*)-**6.11** for similar use (Scheme 6.5), although this method suffered from the need for large volumes of water, and tedious filtrations and extraction.[19]



6.9 6.11

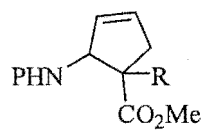
Scheme 6.5. *Reagents and Conditions*: i. Baker's Yeast; ii. HN_3, PPh_3, DEAD; iii. H_2, 10% Pd/C then Boc_2O; iv. a) LiOH, b) 4N HCl, c) Fmoc-Osu, $NaHCO_3$.

Gellman also extended these methods to the preparation of 3-substituted 2-ACPC's **6.12** and **6.13**, for incorporation into short, water-soluble, helical β-peptides (Scheme 6.6).[7]



Scheme 6.6. *Reagents and Conditions*: i. a) $TiCl_4$/TBHP, CH_2Cl_2, b) KO^{t}Bu, benzene, rt; ii. NaN_3/NH_4Cl, MeOH/H_2O, reflux, b) MsCl, pyridine, 0° C, c) LAH, THF, 0° C to rt; iii. a) Boc_2O/NEt_3, MeOH, rt, b) $BF_3.OEt_2$/ROH, CH_2Cl_2, -78° C; iv. a) 10% Pd/C/NH_4HCO_2, MeOH, reflux, b) Jones reagent, acetone, 0° C; v. a) CBz-Cl/NEt_3, CH_2Cl_2, 0° C, b) KCN/18-crown-6, DMSO, 80° C; vi. a) BH_3.THF, THF, rt, b) Boc_2O/NEt_3, MeOH, rt; vii. a) Na/NH_3, -78° C, b) Fmoc-Osu/$NaHCO_3$, acetone/H_2O, rt, c) TEMPO/NACLO, CH_2Cl_2/H_2O, 0° C.

Given the prominent role of ACPCs in synthesis, a need exists for the simple asymmetric synthesis of a range of functionalised ACPCs available from a common low cost precursor. As part of a wider study towards the synthesis of a general class of cyclic β-amino acids via ring-closing metathesis (RCM), we describe here the preparation of a range of cyclopentenyl-based β-amino acids of type **6.14**, that are either unsubstituted, or substituted at the α-position.

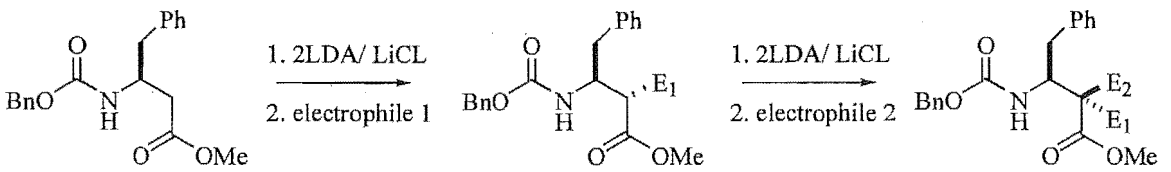


6.14

As for the α-substituted cyclohexenyl-based analogues described in Chapter 5, this second class of compound represents an important addition to the family of cyclic β-amino acids. The constituent olefin of these units is able to be hydrogenated, to give the corresponding saturated analogue, or functionalised to give new and important derivatives.

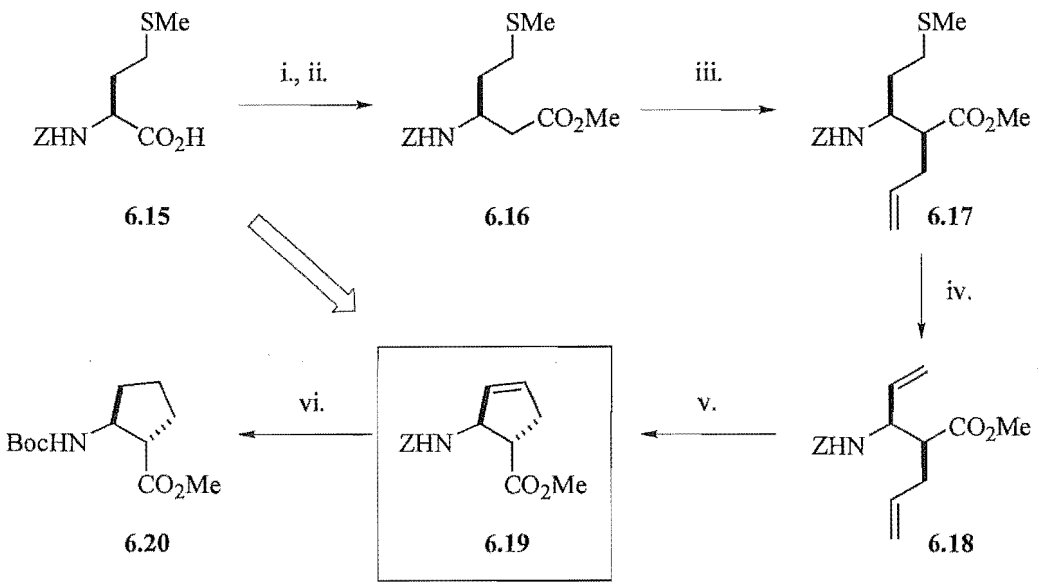
6.2 Synthesis of α-Free Cyclopentenyl-Based Cyclic β-Amino Acids

The synthesis of compounds of type **6.14**, utilised chemistry developed by Podlech and Seebach for the stereoselective alkylation of *N*-Cbz-protected β-amino acids (Scheme 6.7).[20]



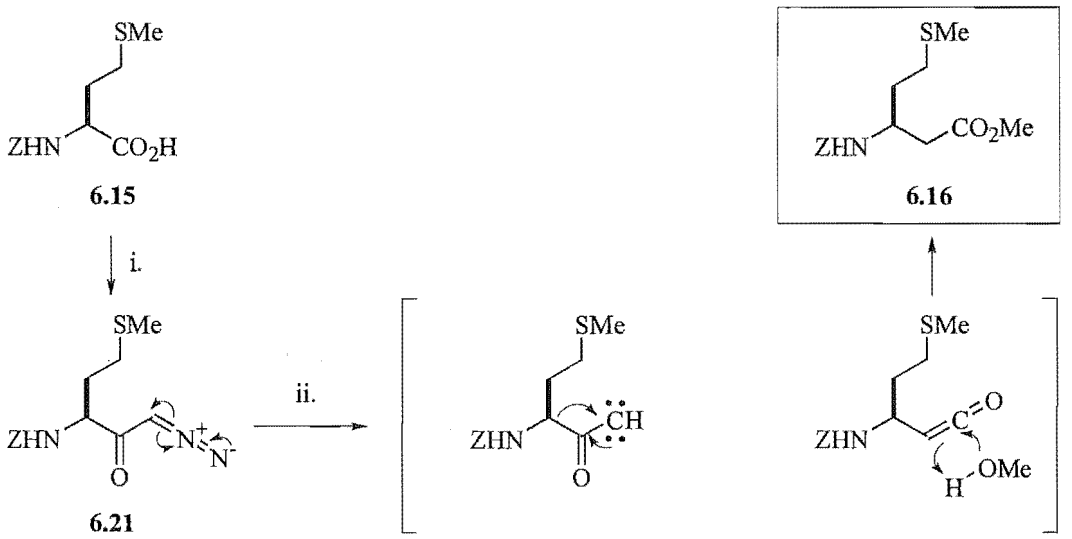
Scheme 6.7. Stereoselective alkylation of β-amino acids developed by Podlech and Seebach

It was anticipated that β-methionine derivatives would undergo oxidative elimination to give dienes suitable for ring-closing metathesis. The proposed synthesis of the α-free aminocyclopentenylcarboxylic acid **6.19**, along with its saturated analogue **6.20**, is described in Scheme 6.8.



Scheme 6.8. *Reagents and Conditions*: i. $ClCO_2Et$, Et_3N, THF, -15°C to 0° C, then CH_2N_2; ii. AgOBz, Et_3N, MeOH, -25° C to rt, 93% 2 steps; iii. 2LDA, LiCl, allyl bromide, THF, -78° C to rt, 16h, 53%; iv. a) H_2O_2-LiOH, AcOH, rt, 4h, quant., b) 200° C, xylene, sealed tube, 76%; v. catalyst **1.42**, benzene, rt, 92%; vi. a) H_2, Pd-C, MeOH, b) Boc_2O, $NaHCO_3$, THF/H_2O (3:1), 75%.

The synthesis began with the preparation of the key precursor *N*-Cbz-β-methionine methyl ester **6.16**. As with the β-amino acid derivatives described in Chapter 5, Arndt-Eistert methodology was adopted for the preparation of **6.16** from (*S*)-*N*-Cbz-methionine. Thus, (*S*)-*N*-Cbz-methionine **6.15** was treated with ethylchloroformate, in the presence of triethylamine, to give the corresponding mixed anhydride, which upon exposure to an ethereal solution of diazomethane gave the diazoketone intermediate **6.21** (Scheme 6.9).



Scheme 6.9. *Reagents and Conditions*: i. a) Et_3N, $ClCO_2Et$, THF, -15° C, 15min, then diazomethane, 0° C; ii) AgOBn, Et_3N, MeOH, -25° C to rt, 93%.

A characteristic singlet at δ_H 5.50ppm, corresponding to the C**H**N_2 proton, signified the successful preparation of this intermediate which was isolated, after purification by silica chromatography, as a yellow oil in quantitative yield. Treatment of diazoketone **6.21** with silver benzoate, at low temperature, in the presence of methanol, with the exclusion of light, resulted in a Wolff rearrangement to form the desired *N*-Cbz-β-methionine methyl ester **6.16** in 93% after purification by silica chromatography.

With the key precursor **6.16** in hand, we set about introducing a substituent at the α-position in an analogous manner to that described by Podlech and Seebach, and previously used in the preparation of equivalent β-allyl glycine derivatives (Chapter 5, Scheme 5 6 and 5.11). Here we were faced with two strategies for the preparation of the key diene **6.18** from **6.16**. These were 1) oxidative elimination of the thiomethyl group of **6.16** first, followed by allylation with allyl bromide to give **6.18** (Strategy A, Figure 6.7), or 2) alkylation of **6.16** with allyl bromide first, followed by oxidative elimination to form **6.18** (Strategy B, Figure 6.7).

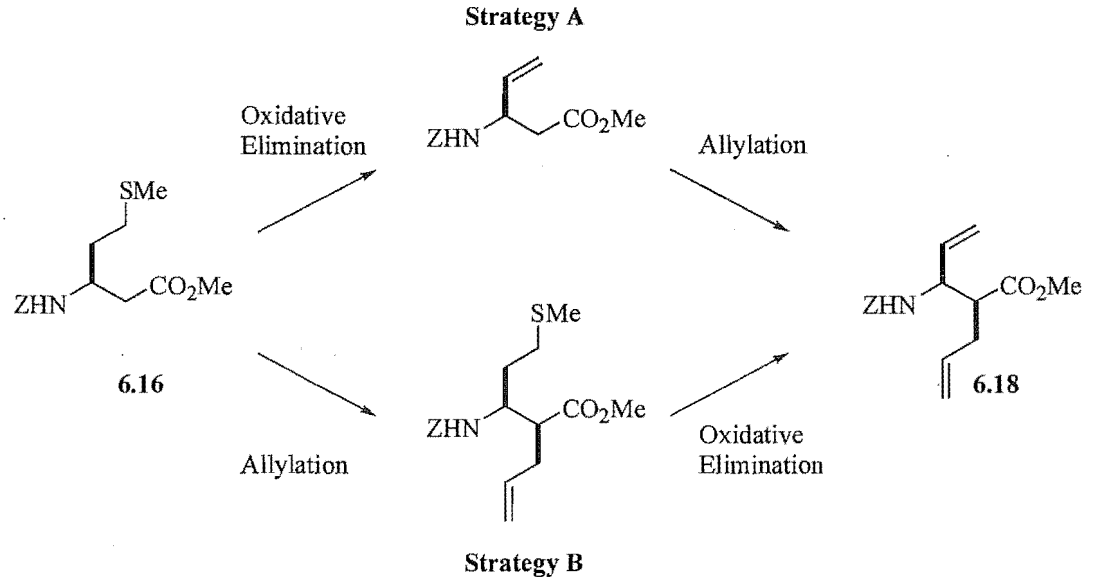
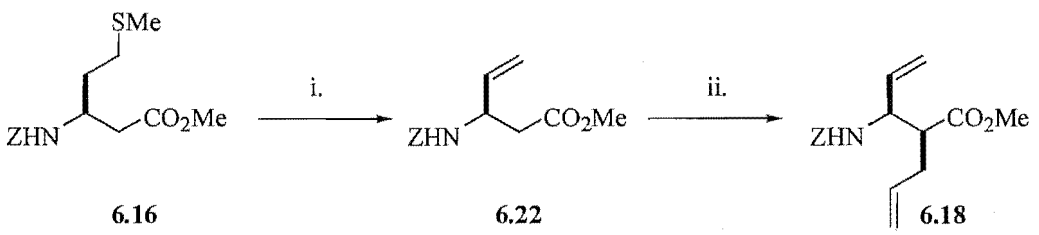


Figure 6.7. Two possible strategies for the preparation of diene **6.18** from **6.16**.

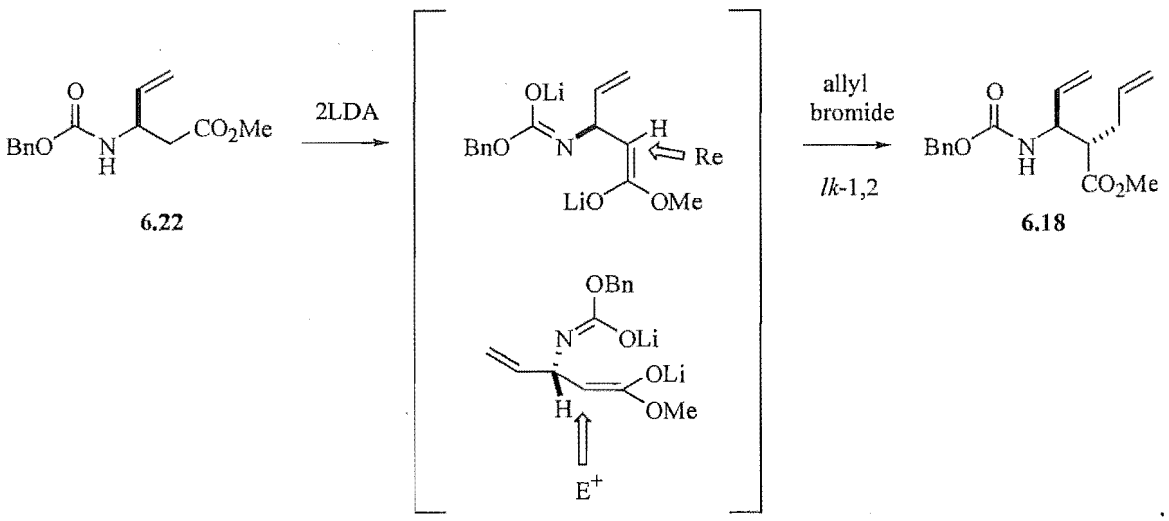
Strategy A was carried out first, with an elimination-allylation sequence being employed for the preparation of **6.18**. Thus, hydrogen peroxide was added to a solution of **6.16**, in acetic acid, to form the corresponding sulphone (Scheme 6.10), which was subsequently dissolved in *degassed* xylene,[a] and sealed in a glass tube under vacuum. The tube was then heated at 200° C for 16 h to give a dark brown solution, which upon purification by chromatography, gave the α-substituted vinyl derivative **6.22**, as a yellow oil in 62% yield over two steps.



Scheme 6.10. *Reagents and Conditions*: i. a) AcOH, H_2O_2, rt, 3h, 99%, b) 200° C, xylene, sealed tube, 62%; ii. . LDA, LiCl, THF, -78° C, allyl bromide, 27%.

[a] A large volume of solvent and gentle heating was required for the sulphoxide to completely dissolve.

Next, **6.22** was deprotonated with LDA, in THF, at -78° C, in the presence of LiCl, and the corresponding enolate alkylated with allyl bromide. It is well documented that these conditions proceed via a doubly lithiated intermediate, with the electrophile adding to the *Re* face of the enolate to give the stereochemistry shown (Scheme 6.11, see also Chapter 5, Scheme 5.8)

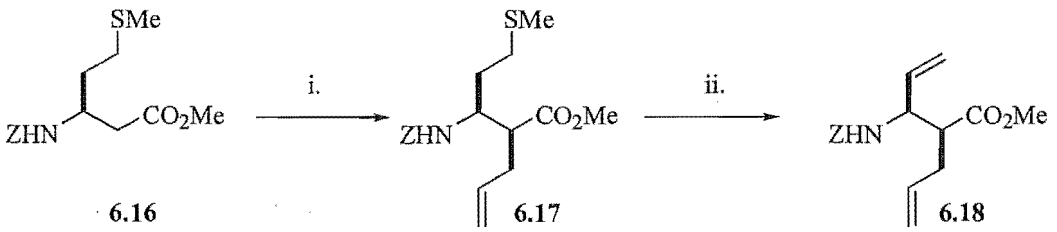


Scheme 6.11. Stereoselective alkylation of **6.22** to give **6.18**

Purification by silica chromatography gave **6.18**, as a single diastereoisomer by ^{1}H NMR, in 27% yield. The poor yield of this reaction may, in part, be due to destabilisation of the vinyl moiety during allylation rather than a lack of specificity, as the minor diasteroisomer of **6.18** was not detected. Measurement of the optical rotation for **6.18** gave an $[\alpha]_D$ = -37° (c=1.0 $CHCl_3$).

Next, strategy B was carried out (Figure 6.7), which involved an allylation-elimination sequence, in an attempt to increase the overall yield of **6.18**. Thus, **6.16** was deprotonated with LDA, in THF at -78° C, in the presence of LiCl, and the corresponding enolate alkylated with allyl bromide (Scheme 6.12). Purification by silica chromatography gave **6.17**, with the stereochemistry shown, as a single diastereoisomer by ^{1}H NMR, in 53% yield. Significantly,

this allylation proceeded in higher yield than in strategy A, indicating that **6.16** was more conducive to alkylation than **6.22**.



6.16 **6.17** **6.18**

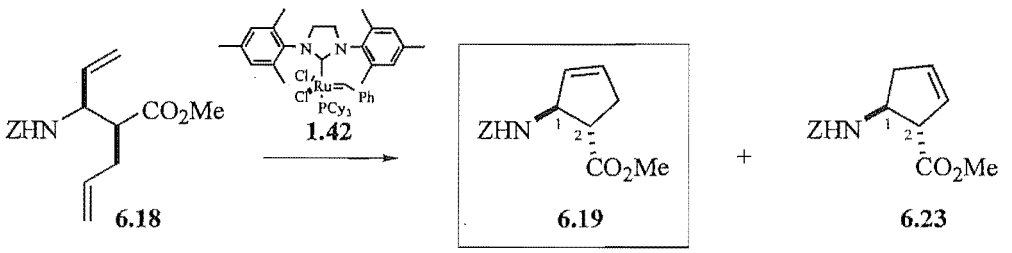
Scheme 6.12. *Reagents and Conditions*: i. LDA, LiCl, THF, -78° C, ally bromide, 53%: ii. a) AcOH, H_2O_2, quant., b) 200° C, xylene, sealed tube, 76%.

Oxidative elimination of the thiomethyl group of **6.17** was then carried out. Hydrogen peroxide was added to a solution of **6.17**, in acetic acid, to form the corresponding sulphone, which was subsequently dissolved in *degassed* xylene,[b] and sealed in a glass tube under vacuum. The tube was then heated at 200° C for 16 h to give a dark brown solution, which upon purification via chromatography, gave the α-substituted vinyl derivative **6.18**, as a yellow oil in 76% yield over two steps. Measurement of the optical rotation for this sample gave a $[\alpha]_D$ = -37°, a value which is in agreement with that observed for **6.18** obtained by strategy A. This led us to consider strategy B as the preferred method of preparation of **6.18** from **6.16**, as the allylation-elimination sequence had a 41% yield over 3 steps, compared with the elimination-allylation sequence of strategy A which had a 17% yield over 3 steps. The difference in yield between the two strategies was reflected in the key allylation step where **6.16** was more conducive to allylation than **6.22**.

With the key diene **6.18** in hand, we demonstrated the basic methodology for the preparation of cyclic β-amino acids, by subjecting diene **6.18** to RCM conditions.[21] To this end, diene **6.18** was treated with catalyst **1.42**, in *dry degassed* benzene, and stirred at reflux overnight (Scheme 6.13). Purification by silica chromatography gave a fraction containing a 1.5:1 ratio

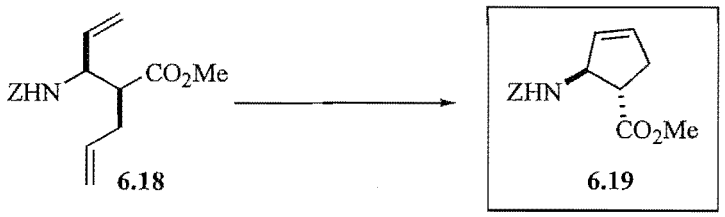
[b] Minimum solvent was required as the sulfoxide was readily soluble.

of regioisomers **6.19** and **6.23**, in 89% yield. Attempts to separate the two regioisomers proved unsuccessful. Assignment of the structures was based upon mass spectrometry, where a single mass of 275.1158 (M^+) was detected, and key 2D COSY correlations between H1 and the olefinic protons of **6.19** and H2 and the olefinic protons of **6.23**.



Scheme 6.13. *Reagents and Conditions*: catalyst **1.42**, benzene, reflux, 89%.

Formation of the minor isomer **6.23** was thought to have been due to isomerism of the double bond of **6.19** brought about by the elevated temperature of the reaction conditions. The reaction was subsequently carried out at room temperature in an attempt to suppress formation of the minor isomer and maximise **6.19**. Thus, diene **6.18** was treated with catalyst **1.42**, in *dry degassed* benzene, and stirred at room temperature overnight. Purification by silica chromatography gave **6.19** exclusively, in 92% yield (Scheme 6.14).



Scheme 6.14. *Reagents and Conditions*: i. catalyst **1.42**, benzene, rt, 93%;

Measurement of the optical rotation for **6.19** gave an $[\alpha]_D$ = +102.3 (c=1.0, $CHCl_3$). The structure of **6.19** was assigned based on key 2D COSY correlations observed for H1, H2, the ring-bound methylene, and the olefinic protons. The ^1H chemical shift data for **6.19**, along

with the molecular connectivity information obtained from a 2D COSY experiment, are shown in Figure 6.8. Exclusive formation of **6.19**, as a result of RCM at room temperature, indicated that isomerization of the double bond of **6.19**, and subsequent formation of **6.23**, had been suppressed.

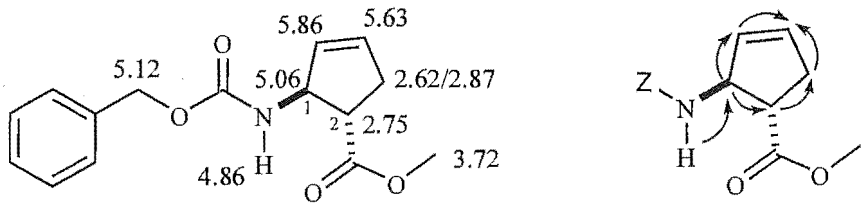
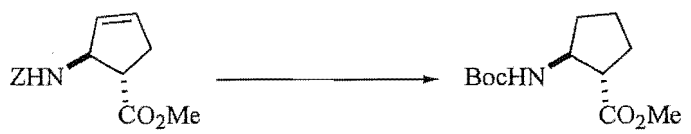


Figure 6.8. Key ^{1}H NMR and 2D COSY data for **6.19**.

The *trans* relative stereochemistry of **6.19** was confirmed by its conversion to the known aminocyclopentanecarboxylic acid **6.20**. Thus, olefin **6.19** was hydrogenated in the presence of 10% palladium-on-carbon, followed by reprotection with Boc_2O, to give the literature compound **6.20** in 75% yield.

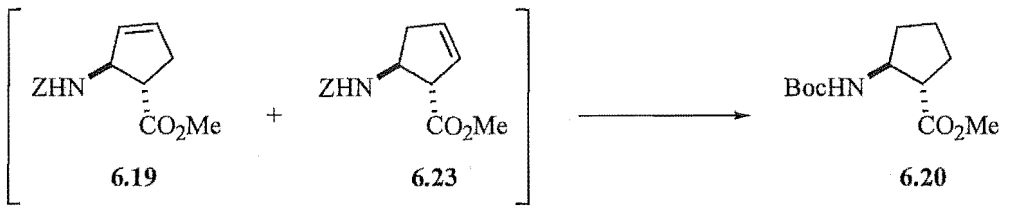


6.19 **6.20**

Scheme 6.15. *Reagents and Conditions*: a) H_2, Pd-C, MeOH, b) Boc_2O, $NaHCO_3$, THF/H_2O (3:1), 75%.

Comparison of the ^{1}H NMR data for **6.20**, with that reported in the literature, confirmed the assigned relative stereochemistry of both the cyclic β-amino acids **6.20** and **6.19**, as well as that of their dienic precursor **6.18**. Measurement of the optical rotation of **6.20** gave an $[\alpha]_D$ = +41.6° (c=0.65, $CHCl_3$), a value in close agreement with that reported in the literature (+44.6°, c=1.3, $CHCl_3$).[13] Significantly, this same conversion was also carried out on the mixture of

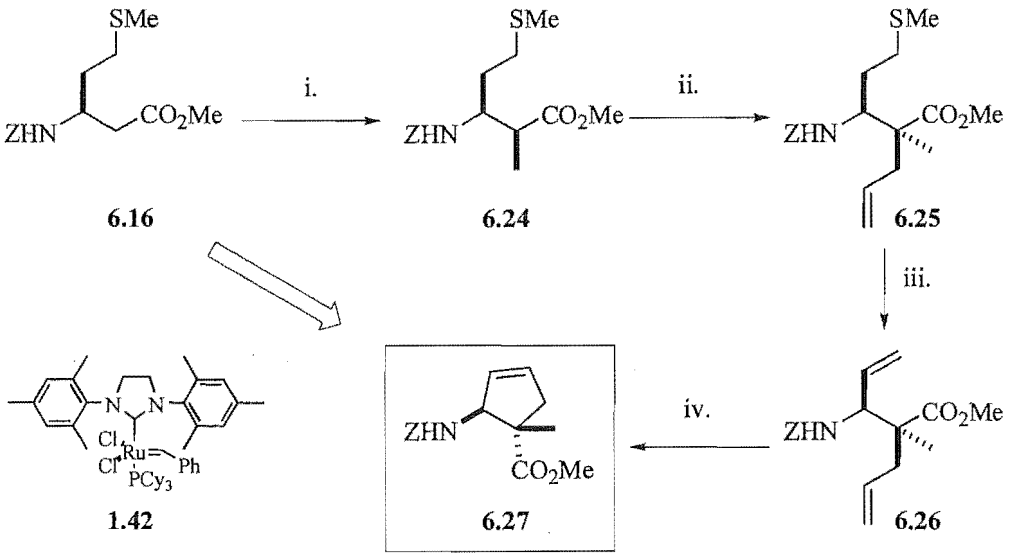
6.19 and **6.23** obtained in Scheme 6.13, resulting in the exclusive isolation of **6.20** (Scheme 6.16). This result further confirmed the relationship of **6.19** and **6.23** as regioisomers.



Scheme 6.16. *Reagents and Conditions*: a) H_2, Pd-C, MeOH, b) Boc_2O, $NaHCO_3$, THF/H_2O (3:1), 75%.

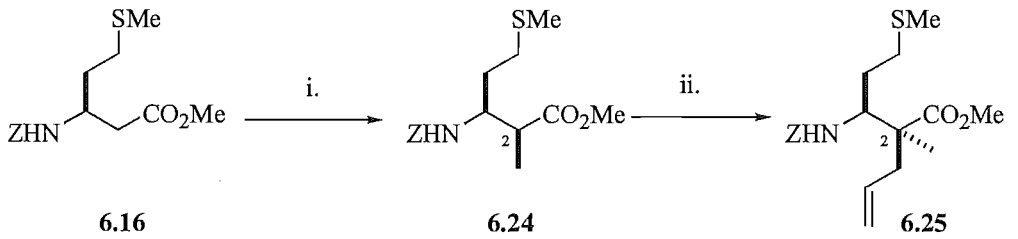
6.3 Synthesis of α-Substituted Cyclopentenyl-Based β-Amino Acids

With this methodology in place we turned our attention to the introduction of a substituent at the α-position. This strategy involved the preparation of suitable dienes, from the common precursor **6.16** (Schemes 6.8 and 6.9), via an alkylation-allylation sequence, similar to that described in Chapter 5 for equivalent cyclohexenyl-based compounds. The proposed synthesis of the *trans* α-substituted cyclopentenyl β-amino acid **6.27** is shown in Scheme 6.17.



Scheme 6.17. *Reagents and Conditions*: i. LDA, LiCl, THF, -78° C, MeI, 92%; ii. LDA, LiCl, THF, -78° C, allyl bromide, 43%; iii. AcOH, H_2O_2, quant., b) 200° C, xylene, sealed tube, 88%; iv. catalyst **1.42**, benzene, rt, 92%.

Here, *N*-Cbz-β-methionine methyl ester **6.16** was used as the common precursor in the preparation of **6.27**, having previously been obtained during the synthesis of the α-free derivative **6.19**. Subsequent alkylation of **6.16**, with methyl iodide in the presence of LDA and LiCl, at -78° C in THF, gave, after purification by silica chromatography, the α-substituted methyl ester **6.24**, as a single diastereoisomer by ^{1}H NMR, in 92% yield (Scheme 6.18). Assignment of the stereochemistry of **6.24** as *syn* was based upon results observed for the preparation of **6.18** and **6.17** (see Schemes 6.11 and 6.12) and upon literature precedent.[20] Measurement of the optical rotation for **6.24** gave an $[\alpha]_D$ = -14.4° (c=1.0, $CHCl_3$).



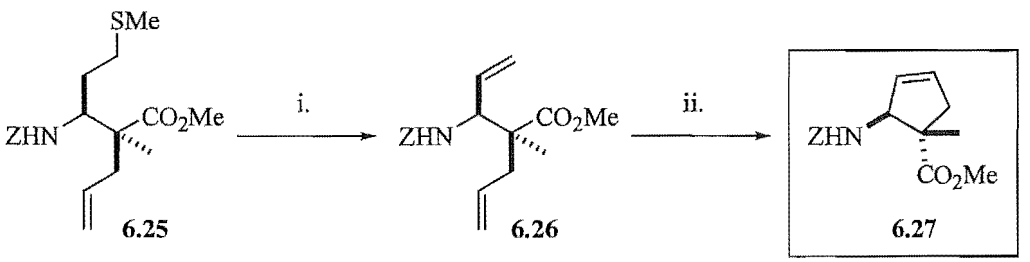
6.16 **6.24** **6.25**

Scheme 6.18. *Reagents and Conditions*: i. LDA, LiCl, THF, -78° C, MeI, 92%; ii. LDA, LiCl, THF, -78° C, allyl bromide, 43%.

An additional alkylation of **6.24**, with allyl bromide, gave the α,α-disubstituted methyl ester **6.25**, as a single diastereoisomer by ^{1}H NMR with the stereochemistry shown, in 43%.yield after purification by silica chromatography. Assignment of stereochemistry for **6.25** was based upon results obtained during the synthesis of equivalent α-substituted cyclohexenyl compounds described in Chapter 5, and upon literature precedent.[20] Measurement of the optical rotation for **6.25** gave an $[\alpha]_D$ = -30.6° (c=1., $CHCl_3$). Note that this second alkylation leads to an inversion of the methyl group at C2, and is the result of the electrophile approaching the planar enolate from the face opposite the benzyloxycarbonylamino group, in a manner similar to that for the preparation of **6.18** (Scheme 6.11, see also Scheme 5.12). It was anticipated that this stereochemistry would lend itself to the formation of the *trans* cyclic β-amino acid upon RCM of the subsequent vinyl diene.

Next, oxidative elimination was carried out on the thiomethyl group of **6.25**, to form the key vinyl diene **6.26** (Scheme 6.19). Hydrogen peroxide was added to a solution of **6.25**, in acetic acid, to form the corresponding sulphone, which was subsequently dissolved in *degassed* xylene,[c] and sealed in a glass tube under vacuum. The tube was then heated at 200° C for 16 h to give a dark brown solution, which upon purification via chromatography, gave the α,α-disubstituted vinyl derivative **6.26**, as a yellow oil in 88% yield over two steps. Measurement of the optical rotation of **6.26** gave an $[\alpha]_D$ = -30.2° (c=1.0, $CHCl_3$).

[c] Minimum solvent was required as the sulfoxide was readily soluble.



6.25 6.26 6.27

Scheme 6.19. *Reagents and Conditions*: i. AcOH, H_2O_2, quant., b) 200° C, xylene, sealed tube, 88%; ii. catalyst **1.42**, benzene, rt, 92%.

Subsequent exposure of diene **6.26** to catalyst **1.42**, in benzene at room temperature, gave, after purification by silica chromatography, the α-methyl cyclic β-amino acid **6.27**, as a single diastereoisomer by ^{1}H NMR, in 92% yield. The assigned *trans* stereochemistry is based on the results of the previous sequence (Schemes 6.8 and 6.15) and literature precedent.[20,22-24]

6.4 Conclusion and Future Work

In summary, we have demonstrated a new and simple procedure for the synthesis of cyclopentenyl β-amino acids, from methionine, based on RCM chemistry. This methodology utilizes an allylation-elimination sequence to prepare the optically active unsubstituted *trans* cyclic β-amino acid (+)-**6.19**, via the simple diene precursor **6.18**. Hydrogenation and reprotection of (+)-**6.19**, gave the known, but important, saturated analogue (+)-**6.20**, the optical rotation of which was in close agreement with that reported in the literature.

We have also demonstrated that an α-substituent, as in **6.27**, can be introduced stereoselectively, by employing an alkylation-allylation sequence, the order of which defines the absolute stereochemistry. This second class of cyclic β-amino acids represents a new and important addition to the family of compounds.

Future work in this area would include the olefinic derivatisation of compounds of this type, with subsequent monomers finding incorporation into oligomers for use in the development of novel foldamers. In addition, the corresponding *cis* isomer of **6.27** could easily be prepared by performing an allylation-alkylation sequence in the preparation of the key dienic precursor.

6.5 References for Chapter Six

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CHAPTER SEVEN

SYNTHESIS OF AMINO ACID-BASED 6-AND 7-MEMBERED RING LACTAMS BY RING–CLOSING METATHESIS

7.1 Introduction

There is increasing interest in the generation of cyclic peptides for use in peptidomimetic research and pharmaceutical development. Medium-sized ring lactams represent suitable fragments in this regard. We describe here the use of RCM methodology for the synthesis of a range of 7-membered ring lactams derived from α-amino acids and β-amino acids.

Medium sized lactams have found widespread use in organic synthesis as key intermediates in the preparation of more complex structures, and as core components of natural products or pharmaceutically important compounds. However, the generation of such nitrogen-containing heterocycles remains a challenge. Compounds incorporating one or more lactam unit have been found to possess a range of interesting structural, biological and pharmaceutical properties. Over the past decade, a substantial number of monolactam-containing natural product examples have been described.[1] These include the cytotoxic bengazoles (Figure 7.1),[2] and related bengamides; the insecticides cripowellin A and B;[3] tuberostemonone;[4] and the fluvirucins B_1 and B_2, isolated in 1990 as a new class of antifungal agents with activity against the influenza A virus.[5]

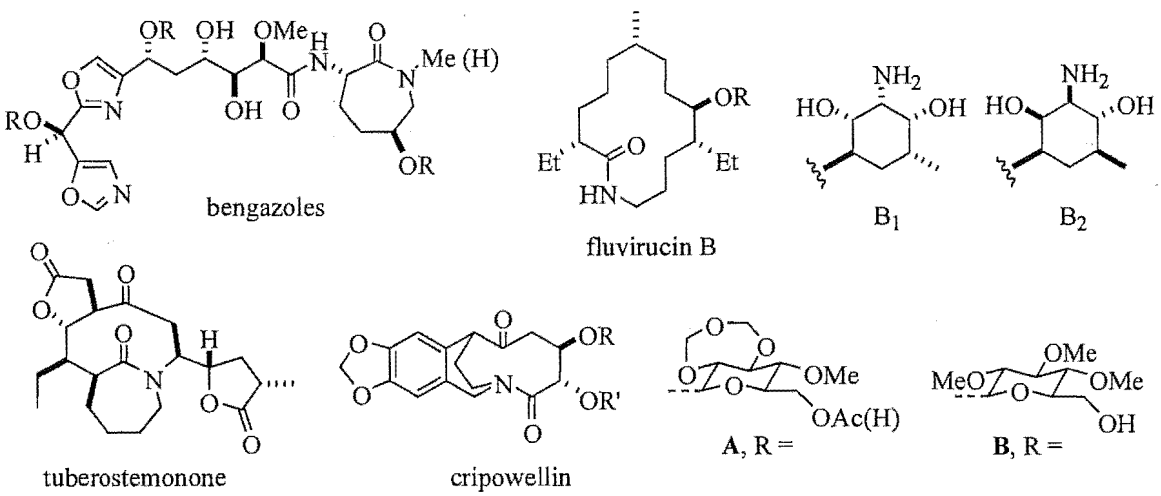


Figure 7.1. Lactam-containing natural products isolated in the 1990's

In addition, lactams have been used in peptidomimetic design to construct such structural motifs as artificial turns or hairpins, or used to stabilise a bioactive conformation through the introduction of a rigid ring. Among the latter, Freidinger lactams have proved the most generally useful in drug design.[1,6] A Freidinger lactam is derived from a peptide in which the α-position of a chosen amino acid residue has been connected to a downstream amide nitrogen through the addition of a carbon bridge (Figure 7.2).

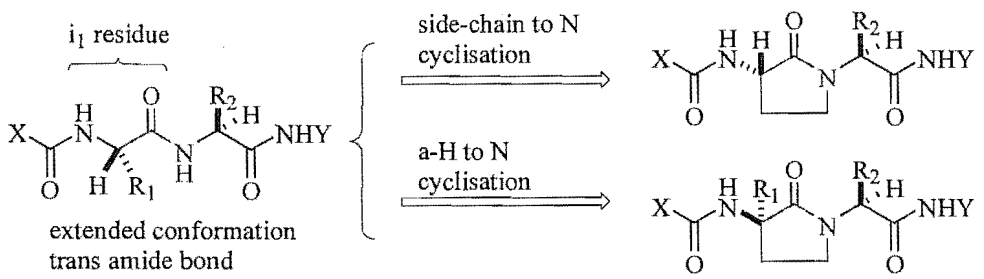


Figure 7.2. Cyclisations between the i_n and i_{n+1} residues, resulting in Freidinger lactams

A pivotal paper in this field published by Freidinger concerned the synthesis of a potent analogue of the hypothalamic hormone luteinizing-hormone-releasing hormone (LHRH, also known as gonadotropin-releasing hormone or GRH).[7] The lactam-containing analogue **7.1** was designed to mimic the Tyr-Gly-Leu-Arg type II β-turn in the bioactive conformation of the molecule, and was found to be 2.4 times as potent as LHRH in *in vivo* studies (Figure 7.3). Lactams of this type were subsequently used to target other biological processes, with **7.2** found to inhibit the formation of methane in sheep stomach fluid.[8,9a]

[a] This compound may prove useful in NZ's current political climate i.e. animal gas tax.

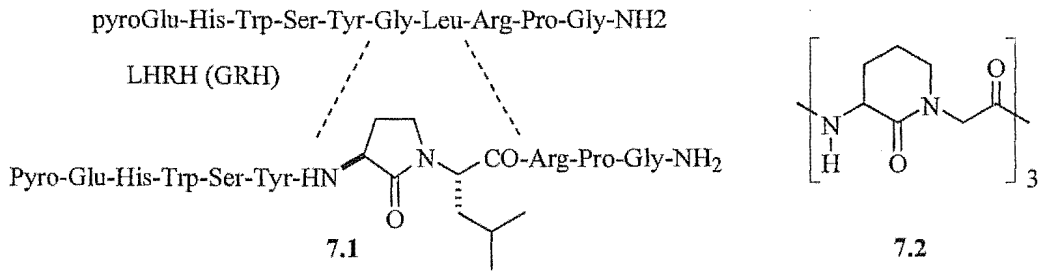


Figure 7.3. Examples of lactam-containing peptidomimetics

Other recent examples include amnesia-reversal compounds of type **7.3** and **7.4**,[10] serine protease inhibitors of type **7.5**,[11] and hepatitis C virus NS3 protease inhibitors of type **7.6**.[12]

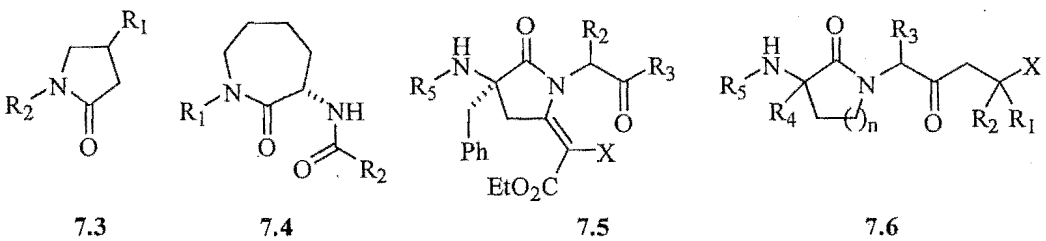


Figure 7.4. Recent examples of lactam peptidomimetics

Various bicyclic lactam scaffolds have been developed. These include the potent ACE inhibitors **7.7** (cilazaprilat) and **7.8**, cyclophilin inhibitors of type **7.9**,[13] and β-strand templates of type **7.10** that have recently been incorporated into potent inhibitors of a range of serine proteases, including thrombin (see Chapter 4).[14]

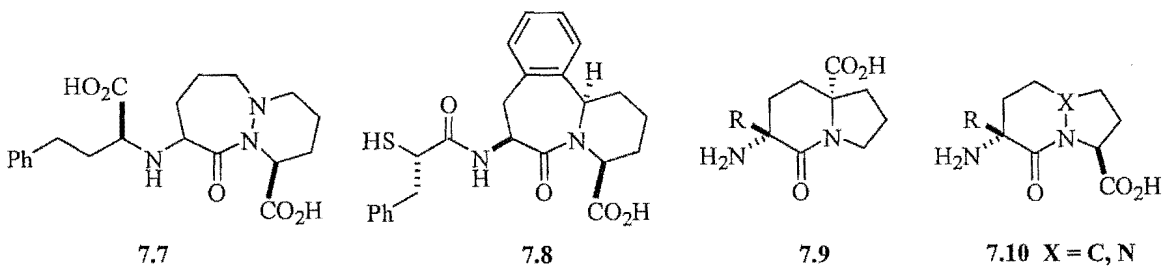


Figure 7.5. Bicyclic lactam peptidomimetics

The synthesis of lactam-based peptidomimetics has thus become the subject of much interest. The original route to Freidinger lactams remains one of the simplest and most useful methods for the preparation of γ-lactam peptidomimetics. The sulfide moiety of a methionine-containing dipeptide is converted to an appropriate leaving group (i.e $^{+}SMe_2$), and subsequently cyclized under basic conditions (Figure 7.6).[7] Allyl groups and alkyl halides have also proven successful in this regard

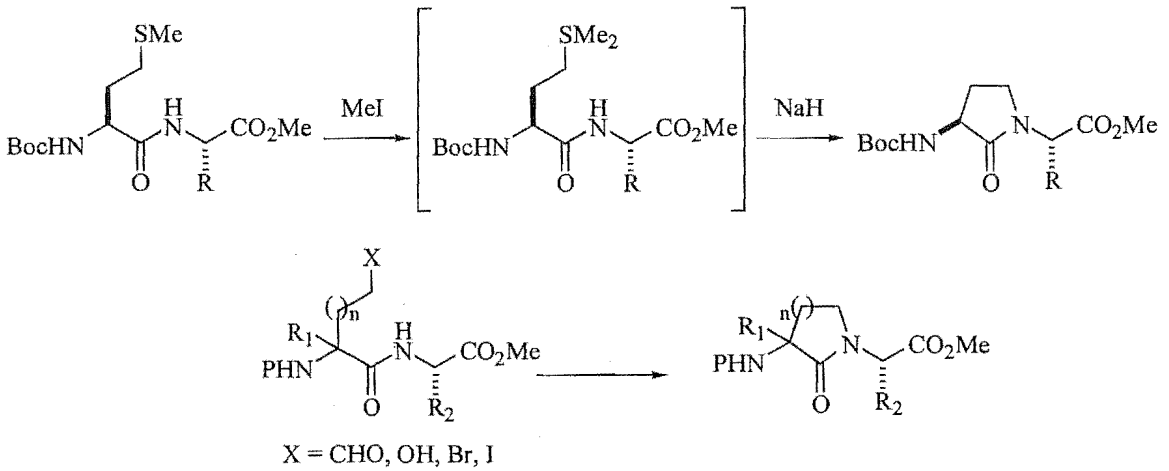
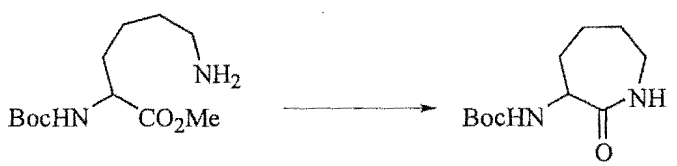


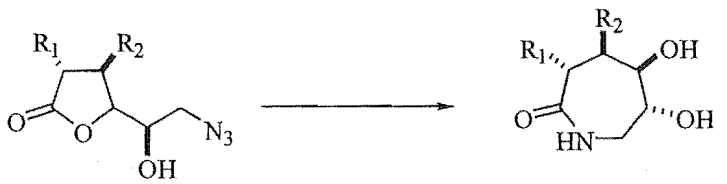
Figure 7.6

An alternative method for the generation of lactams is through activation of the carboxylic acid, and subsequent intramolecular cyclisation, of amino esters. An example is the lactamisation of *N*-Boc-lysine methyl ester using PyBop (Scheme 7.1).[15] This can also be achieved using the equivalent free acid under standard peptide coupling conditions.



Scheme 7.1. *Reagents and Conditions*: PyBop, $NaHCO_3$, DMF, 85-90%.

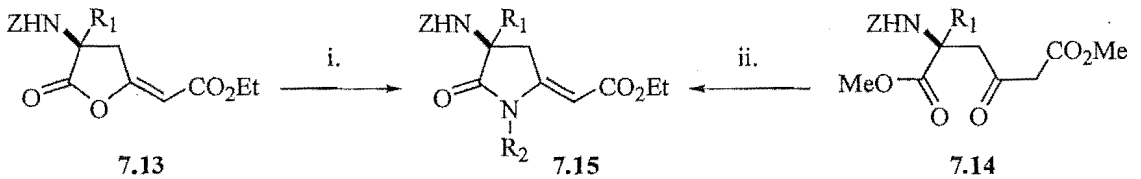
A combination of N and C activation has been used to generate 7-membered ε-caprolactams of type **7.12** from a range of *D*-galactono-1,4-lactone-derived terminal azides **7.11** (Scheme 7.2).[16-18]



7.11 **7.12**

Scheme 7.2. *Reagents and Conditions*: H_2/Pd (black), 65-98%

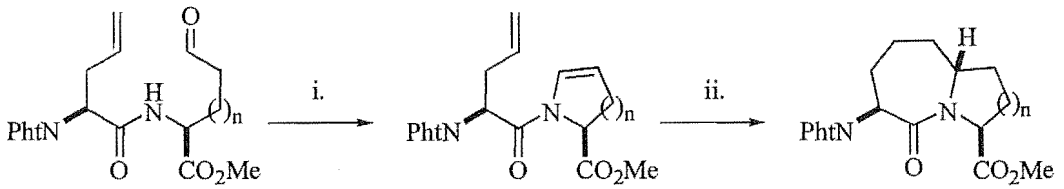
Research from this laboratory has described the preparation of substituted enamino ester of type **7.15** from either enol lactones **7.13**,[11] or β-keto esters **7.14** (Scheme 7.3).[19]



7.13 **7.15** **7.14**

Scheme 7.3. *Reagents and Conditions*: i. a) R_2NH_2.HCl, Et_3N, CH_2Cl_2, rt, b) PTSA, 1,2-DCE, reflux; ii. a) R_2NH_2, 1,2-DCE, reflux, b) 150° C, 1mm, R=H 70%, R=Bn 43%.

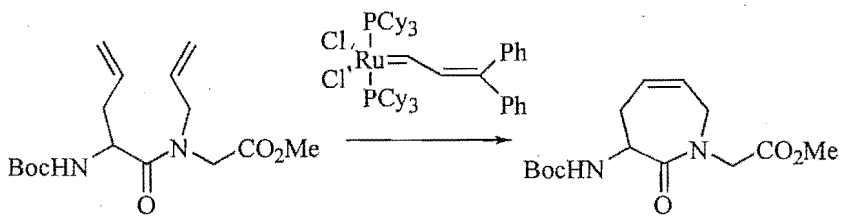
Bicyclic compounds of type **7.17** have been prepared through acid-mediated cyclisation of acyclic dipeptides **7.16** (Scheme 7.4).[20] Various other methods for lactam formation have been reviewed.[1]



7.16 7.17

Scheme 7.4. *Reagents and Conditions*: i. TFA/CH_2Cl_2, reflux, 98%; ii.a) TfOH, Tf_2O, CH_2Cl_2, rt, 21h, b) NaI, MeC(O)Et, c) $(TMS)_3SiH$, PhMe, reflux, n=1 71%, n=2 68%.

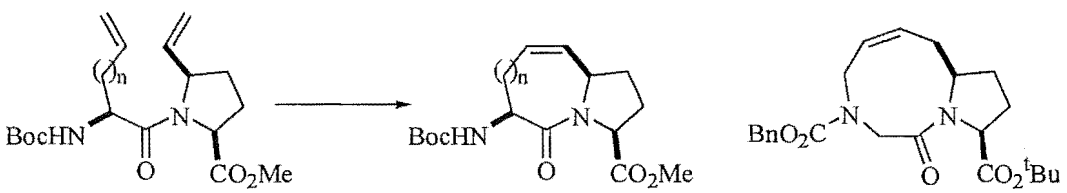
Recently, RCM methodology has been used to prepare medium-sized ring lactams. The ability of this technique to access a range of ring sizes has made RCM of particular interest in this regard. Grubbs *et al*, among others, have demonstrated the application of RCM methodology towards the synthesis of a range of cyclic amino acids including the 7-membered example **7.18** (Scheme 7.5).[21]



7.18

Scheme 7.5. *Reagents and Conditions*: 5 mol%, $CHCl_3$, rt, 52%.

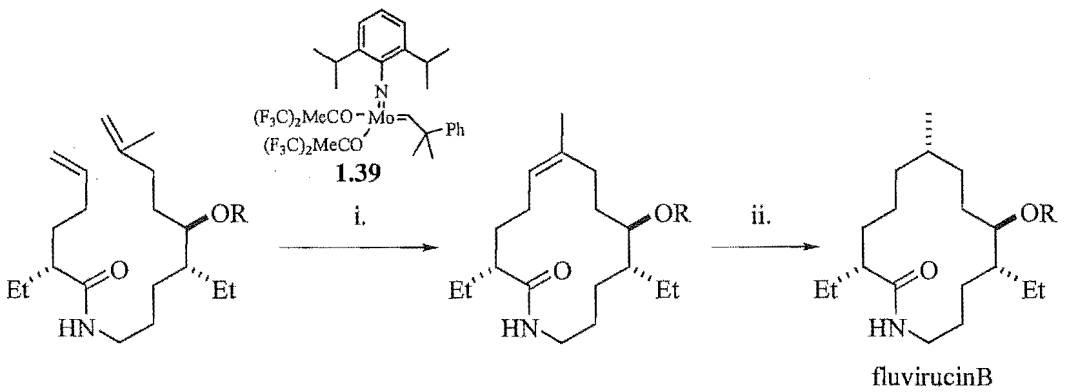
Moeller *et al* used RCM in the preparation of bicyclic lactams of type **7.19**,[22] while Brimble *et al* used similar methodology to access compounds of type **7.20** (Scheme 7.6).[23]



n =0,1 7.19 7.20

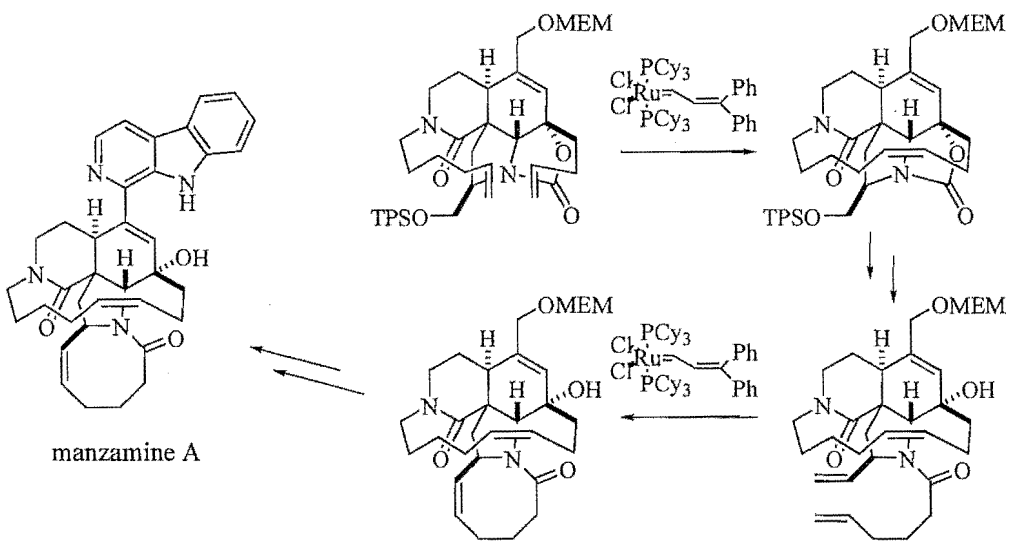
Scheme 7.6. *Reagents and Conditions*: **1.40**, CH_2Cl_2, reflux, 90%.

RCM methodology has been applied in the synthesis of a number of lactam-containing natural products. An example is the use, by Hoveyda *et al*, of Schrock's catalyst **1.39** in the enantiolective synthesis of fluvirucin B_1 (Scheme 7.7).[24]



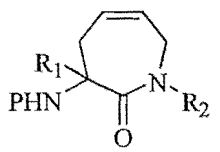
Scheme 7.7. *Reagents and Conditions*: i. 20 mol% **1.39**, 90%; ii. H_2/Pd-C, 84%.

Another impressive example is the generation of advanced intermediates en route to manzamine A. Here, an 8-membered lactam and a 13-membered lactam are introduced under mild conditions at two different stages of the synthesis (Scheme 7.8).[25,26]

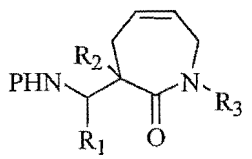


Scheme 7.8. Use of RCM in the synthesis of manzamine

RCM has increasingly found use in the synthesis of a range of novel lactams and as a result we desired to extend its application in the area of peptidomimetics to the preparation of compounds of type **7.21** and **7.22**. The synthesis of α-unsubstituted examples of type **7.21** has previously been described by Grubbs *et al* (refer Scheme 7.5), however a need still exists for the development of a methodology for the incorporation, with stereocontrol, of a substituent at the α-position. Compounds of this type were unreported in the literature at the commencement of this work. Consequently, analogues of type **7.22** represent an extension of this methodology towards the synthesis of lactams incorporating functionalised β-amino acids.



7.21

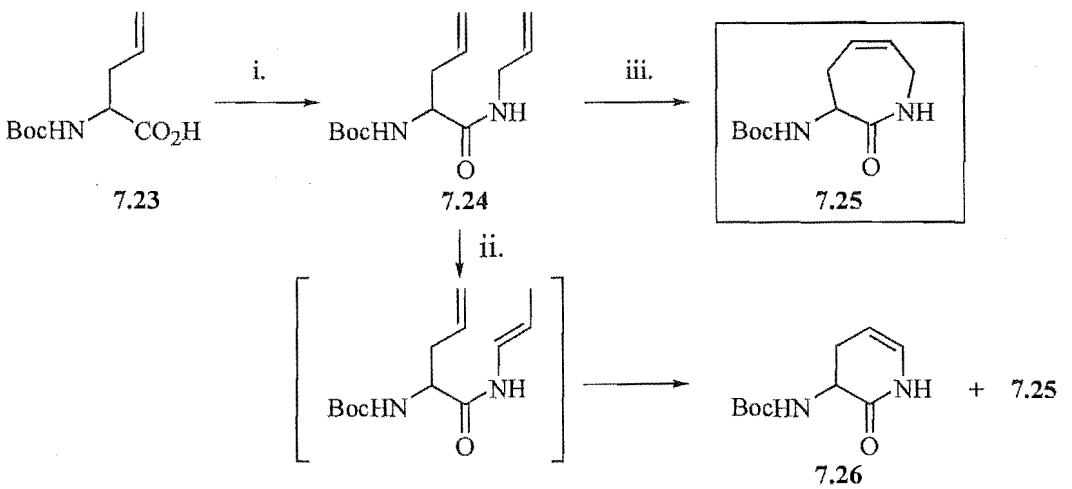


7.22

7.2 Lactams from α-Amino Acids

In order to develop a methodology for the synthesis of α-substituted lactams of type **7.21**, we began by preparing the allylglycine derivative **7.25**, an analogue that is unsubstituted at the α-position (Scheme 7.9). In a manner similar to that described by Grubbs in Scheme 7.5, (+/-)-N-Boc-allyl glycine **7.23** was condensed with allylamine under standard EDCI/HOBt/DIEA coupling conditions, to give, after purification by silica chromatography, diene **7.24** in 92% yield. Exposure of diene **7.24** to RCM conditions, in the presence of catalyst **1.42**, at 100° C, gave a crude mixture of the 7-membered lactam **7.25**, and the 6-membered lactam **7.26**, in a ratio of approximately 1:1 by ^{1}H NMR. Purification by silica chromatography allowed separation of the lactams, with **7.25** and **7.26** isolated in 45% and 46% respectively. This is the first report of a ring-contraction being observed during a reaction of this type, with the formation of **7.26** suggesting that a thermally induced migration of the terminal olefin had taken place prior to RCM. Subsequently, diene **7.24** was subjected to a second RCM reaction,

this time at a lower temperature of 85° C. The result was the exclusive formation, by ^{1}H NMR, of **7.25**, which was isolated, after purification by silica chromatography, in 89% overall yield.



Scheme 7.9. *Reagents and Conditions*: i. EDCI, HOBt, DIEA, allylamine, CH_2Cl_2, 92%; ii. catalyst **1.42**, benzene, 100° C, **7.25** 45%, **7.26** 46%; iii. catalyst **1.40** or **1.42**, benzene, 85° C, **7.25** 89%.

The ^{1}H chemical shift and mass spectrometry data for **7.25** and **7.26**, along with the molecular connectivity information obtained from respective 2D COSY experiments, are shown in Figure 7.7.

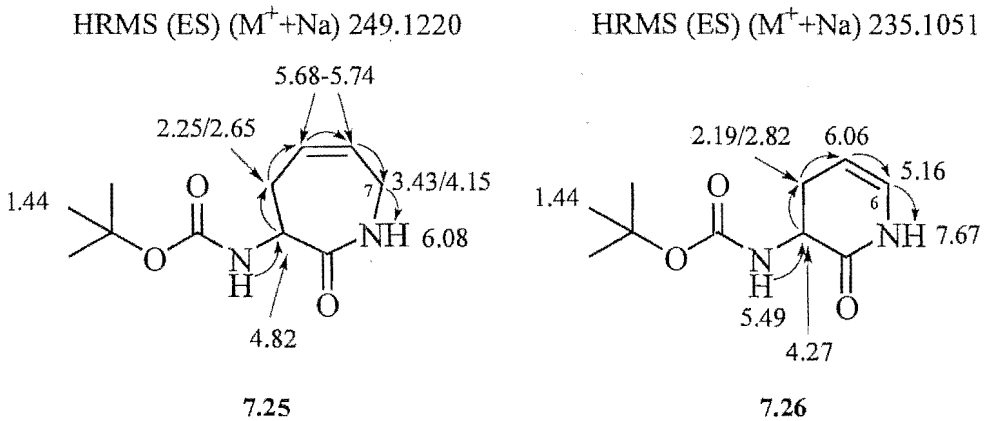
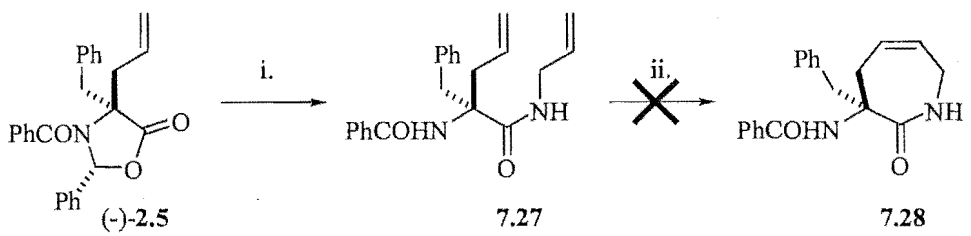


Figure 7.7. Key ^{1}H NMR and mass spectrometry data for **7.25** and **7.26**.

Key ^{1}H NMR results for **7.25** included correlations between the C-7 methylene protons and the adjacent C-6 olefinic proton and lactam NH. Key results for **7.26** included a correlation between the lactam NH and the adjacent olefinic proton at C-6. Subsequent analysis of **7.25** and **7.26** by mass spectrometry confirmed these assignments.

Having successfully prepared α-free lactams of type **7.21**, we next turned our attention to the preparation of compounds of type **7.21** where a substituent is introduced at the α-position. The proposed synthesis of **7.28**, is shown in Scheme 7.10, with oxazolidinone chemistry pioneered by Seebach used to prepare the α,α-disubstituted oxazolidinone **2.5**, and amine hydrolysis, followed by RCM, used to effect formation of the lactam. The α,α-disubstituted oxazolidinone **2.5** provided a convenient, and optically active starting material for this synthesis, having found previous use in the syntheses of the tetrahydropiperidine **2.9** (see Schemes 2.2 and 2.4 in Chapter 2) and the bicyclic template **4.14** (see Schemes 4.2 and 4.3 in Chapter 4) This compound was previously demonstrated as having an enantiomeric excess >95% (refer Chapter 2.6), with the absolute stereochemistry, defined by the amino acid from which it is derived, having been confirmed by X-ray crystallography (Figure 2.10). Thus, n-BuLi was stirred for 5min with allylamine in THF at -78° C, following which a solution of **2.5** in THF was slowly added. The solution was then stirred at rt overnight following which purification by silica chromatography gave diene **7.27** in 91%.



(-)-2.5 7.27 7.28

Scheme 7.10. *Reagents and Conditions*: i. n-BuLi, allylamine, THF, -78° C to rt, 91%; ii. catalyst **1.42**.

Crystals of **7.27** were obtained and the solid-state structure was subsequently determined at 566° K and suitably refined A perspective drawing of **7.27**, with atom labelling, is shown in Figure 7.8.

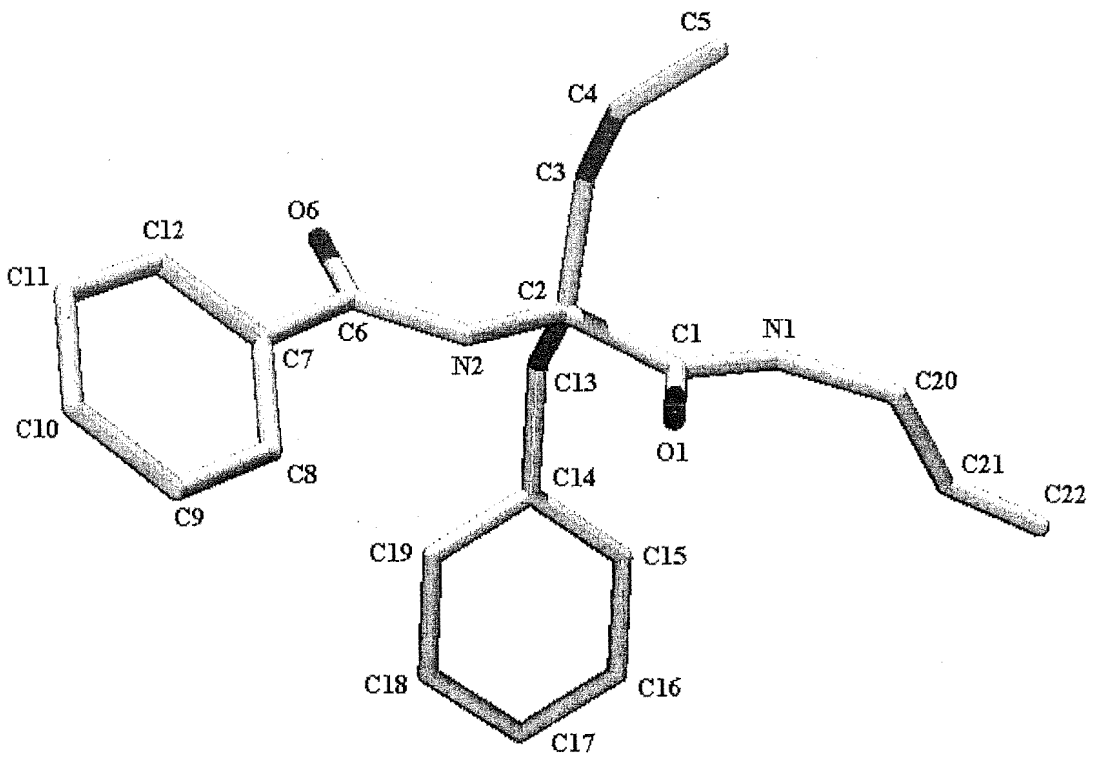
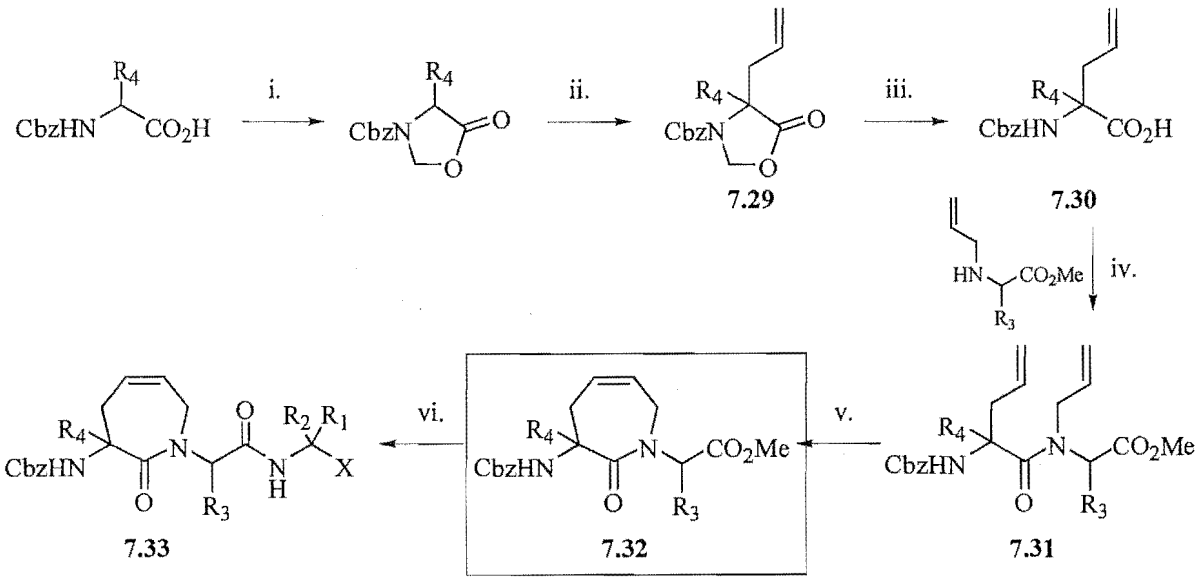


Figure 7.8. Solid-state structure of diene **7.27**.

Analysis of the solid-state structure of **7.27** shows the diene in an extended conformation, with the terminal olefin adopting a *trans* relationship across the C1-N1 amide bond with respect to the rest of the molecule. The stereochemistry at the α-carbon (C2) of **7.27** was assigned as *R*, based on the previous assignment of **2.5** (refer Chapter 2), with the $P2_12_12_1$ space group assigned to the crystal indicating the molecule to be chiral.

Next, we subjected diene **7.27** to RCM in an attempt to prepare the α-substituted lactam **7.28**. However, RCM did not occur under a range of conditions (CH_2Cl_2 or benzene, rt or reflux), or

with a variety of catalysts (**1.40**, **1.41**, or **1.42**), and in all cases diene **7.27** was recovered in quantitative yield. This contrasts the α-unsubstituted example **7.24** (Scheme 7.9) that readily underwent RCM, a result that led us to consider that substitution of the allyl amide nitrogen may be necessary to invoke RCM in the α-substituted case. While this was being contemplated, Priestley and Decicco at Bristol-Myers Squibb duly patented a similar methodology for the racemic synthesis of novel lactams of type **7.33** as inhibitors of hepatitis C virus NS3 protease (Scheme 7.11).[12]

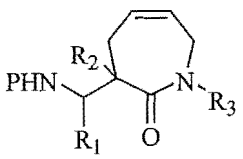


Scheme 7.11. Priestley and Decicco's synthesis of α-substituted cyclic lactams. *Reagents and Conditions*: i. paraformaldehyde, TsOH, benzene; ii. KHMDS, allyl bromide; iii. NaOH, MeOH/H_2O; iv. PyAOP, DIEA, DMF; v. catalyst **1.40**; vi. a) LiOH, b) $H_2NCR_1R_2X$.

Here the racemic oxazolidinone **7.29** is hydrolysed to the acid **7.30**, which is coupled to a substituted-*N*-allyl amino acid methyl ester using activating reagents suitable for hindered peptide coupling reactions, to afford dipeptide **7.31**. RCM with Grubbs ruthenium catalyst **1.40**, results in the formation of lactam **7.32**. This result is consistent with our suggestion that α-substituted dienes of this type require a substituent on the allyl amide nitrogen before RCM can occur.

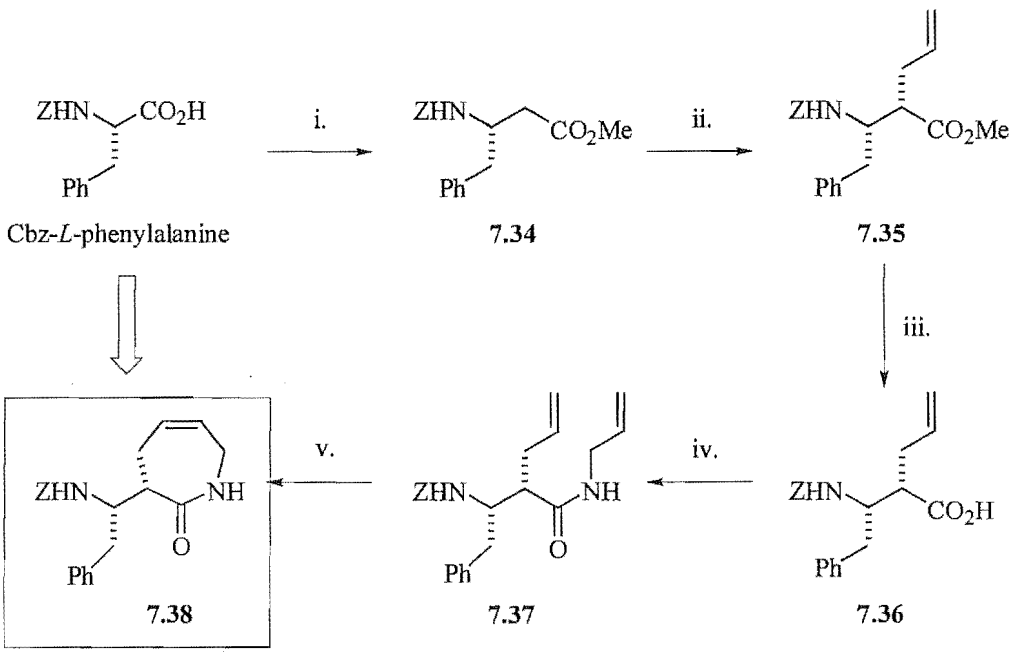
7.3 Lactams from β-Amino Acids

Increasing interest has recently focused on the incorporation of β-amino acids into peptidomimetics due to their enhanced biological properties and increased biostability. However, the incorporation of β-amino acids into cyclic lactams has remained largely unexplored in this regard, and as such we set out to develop a methodology whereby lactams of type **7.22**, that were either unsubstituted or substituted at the α-position, could be prepared from a common precursor.



7.22

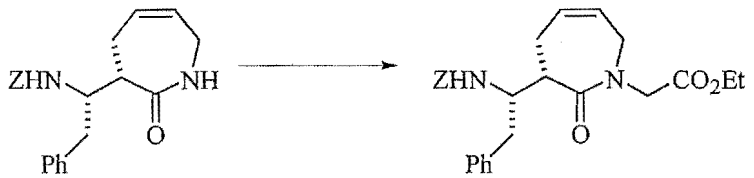
The proposed synthesis of the α-unsubstituted β-phenylalanine-derived lactam **7.38** is shown in Scheme 7.12. It was envisaged that stereoselective allylation of Cbz-protected methyl 4-phenyl-3-aminobutanoate **7.34**, to give **7.35**, followed by hydrolysis and coupling of allyl amine would give diene **7.37**, that would subsequently undergo RCM to form the 7-membered cyclic lactam **7.38**.



Scheme 7.12. *Reagents and Conditions*: i. a) Et_3N, $ClCO_2Et$,THF, -15° C, 15min, b) CH_2N_2, Et_2O, 0° C to rt, c) AgOBz, Et_3N, MeOH, -25° C to rt,, 96% 3 steps; ii. LDA, LiCl, allyl bromide, THF, -78° C, 64%; iii. NaOH, MeOH, 99%; iv. EDCI, HOBt, DIEA, allylamine, CH_2Cl_2, 74%; v. catalyst **1.42**, benzene, 50° C, 89%.

The synthesis follows a similar strategy to that used in the preparation of cyclic β-amino acids described in Chapters 5 and 6 (Schemes 5.6 and 6.8), with Cbz-protected methyl 4-phenyl-3-aminobutanoate **7.34** being prepared, using Arndt-Eistert methodology, in a manner similar to that of **5.25** (Scheme 5.7) and **6.16** (Scheme 6.9).[27] Thus, *N*-Cbz-*L*-phenylalanine was reacted with triethylamine and ethylchloroformate, to give the corresponding mixed anhydride, which, upon treatment with diazomethane, gave a diazoketone intermediate that exhibited a characteristic singlet at δ_H 5.23ppm, corresponding to the C**H**N_2 proton. Exposure of the diazoketone to silver benzoate, at low temperature, in the presence of methanol, with the exclusion of light, resulted in a Wolff rearrangement to form the desired Cbz-protected methyl 4-phenyl-3-aminobutanoate **7.34**, which was isolated after silica chromatography in 96% yield over 3 steps. With this key β-amino acid in hand we set about introducing a substituent at the α-position, using a general method previously described by Podlech and Seebach.[27] Thus,

7.34 was deprotonated with LDA, in THF at -78° C, in the presence of LiCl, and the corresponding enolate was alkylated with allyl bromide. Purification by silica chromatography gave **7.35**, as a single diastereoisomer by ^{1}H NMR, in 64% overall yield. Hydrolysis of **7.35** with NaOH gave **7.36**, which upon coupling with allylamine under standard EDCI/HOBt/DIEA conditions gave, after purification by silica chromatography, diene **7.37** in 74% yield. Subsequent treatment of diene **7.37** with catalyst **1.42**, in *dry degassed* benzene at 50° C for 2h, gave, after purification by silica chromatography, **7.38** in 93% yield. This is consistent with results obtained for the equivalent α-amino acid derived compound **7.25** (Scheme 7.9). To illustrate that it was possible to incorporate compounds of this type into a peptide sequence, **7.38** was subsequently alkylated on the lactam nitrogen, with ethyl bromoactetate in the presence of NaH, to give, after purification by silica chromatography **7.39** in 92% yield (Scheme 7.13). This result represents a new and novel application of functionalised β-amino acids in the synthesis of cyclic lactams, with RCM methodology used as the key step in the formation of the heterocycle.



7.38 **7.39**

Scheme 7.13. Reagents and Conditions: NaH, $BrCH_2CO_2Et$, CH_3CN, rt, 24h, 92%.

Key ^{1}H NMR data, and 2D COSY connectivity correlations, confirming the structural assignment of **7.39**, are shown in Figure 7.9. Correlations observed between the C-7 methylene protons and the adjacent H-6 olefinic proton established connectivity within the lactam ring, with correlations between the H-3 α-proton and the adjacent β-proton allowing assignment of the exocyclic resonances.

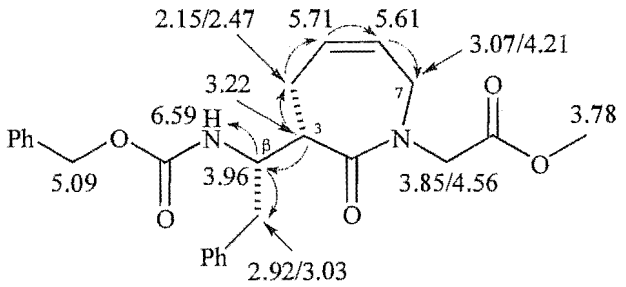
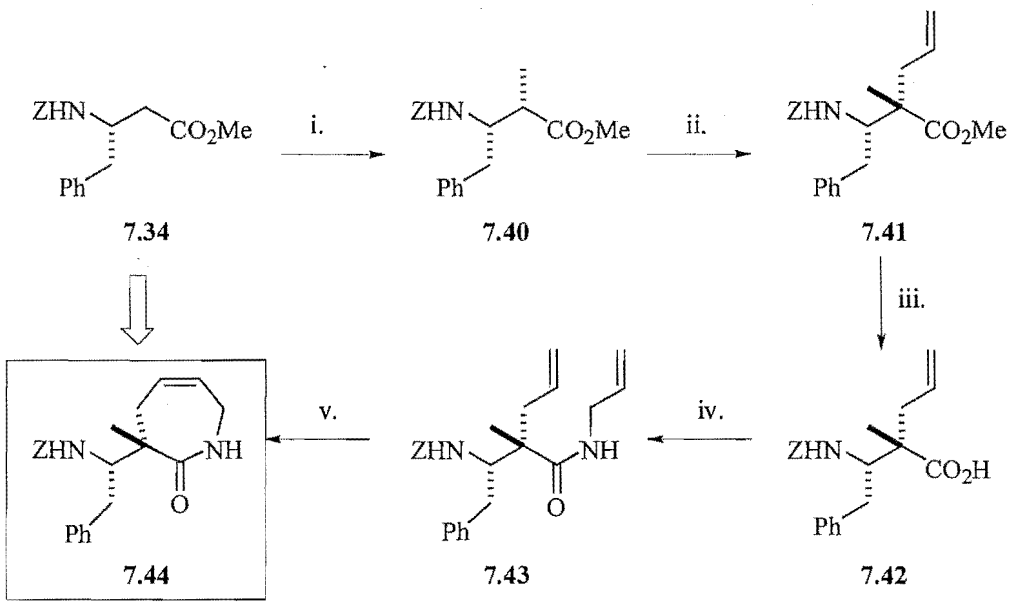


Figure 7.9. Key ^{1}H NMR data for **7.39**.

We next set about incorporating a substituent at the α-position of **7.38** via the method shown in Scheme 7.14. It was envisaged that an alkylation – allylation sequence would be carried out on the common precursor **7.34**, to give **7.41** in a manner similar to that used in the preparation of the α-substituted cyclic β-amino acids **5.34** (Scheme 5.11) and **6.27** (Scheme 6.17) described in Chapters 5 and 6. Hydrolysis of **7.41**, followed by coupling of allylamine would give diene **7.43**, which would then undergo RCM to give the α-substituted 7-membered ring lactam **7.44**.



Scheme 7.14. *Reagents and Conditions*: i. LDA, LiCl, MeI, THF, -78° C, 84%; ii. LDA, LiCl, allyl bromide, THF, -78° C, 39%; iii. NaOH, MeOH, 99%; iv. EDCI, HOBt, DIEA, allylamine, CH_2Cl_2, 74%; v. catalyst **1.42**, benzene.

Thus, alkylation of the common precursor **7.34** with methyl iodide, in the presence of LDA and LiCl, gave, after purification by silica chromatography the α-methyl substituted methyl ester **7.40**, in 84% yield as a single diastereoisomer by ^{1}H NMR. The stereochemistry of **7.40** was assigned as *syn* based upon literature precedent and analogous compounds prepared in Chapters 5 and 6 (refer Schemes 5.6 of Chapter 5 and Schemes 6.8 in Chapter 6).[27] Compound **7.40** was subsequently alkylated a second time, with allyl bromide, to give, after purification by silica chromatography, the α,α-disubstituted alkene **7.41**, in 39% yield as a single isomer by ^{1}H NMR. The stereochemistry depicted is based upon both literature precedent,[27] and analogous compounds prepared in Chapters 5 and 6 (refer Scheme 5.11 in Chapter 5 and Scheme 6.16 in Chapter 6). X-ray crystal analysis of **7.43**, a derivative of **7.41**, subsequently confirmed this assignment. Hydrolysis of **7.41** with NaOH gave **7.42**, which upon coupling with allylamine under standard EDCI/HOBt/DIEA conditions gave, after purification by silica chromatography, diene **7.43** in 74% yield.

The solid-state conformation of **7.43** was subsequently determined to allow confirmation of the relative stereochemistry. X-ray crystallographic analysis revealed **7.43** to have crystallised in the space group $P2_12_12_1$, with 3 independent molecules in the asymmetric unit. The absolute stereochemistry at C3 of **7.43** was assigned as *S* based upon the stereochemistry of (*S*)-phenylalanine from which it was derived. This allowed the relative stereochemistry of **7.43** to be confirmed as that shown in Scheme 7.14. A perspective drawing of one of the molecules of **7.43**, with atom labelling, is shown in Figure 7.10.

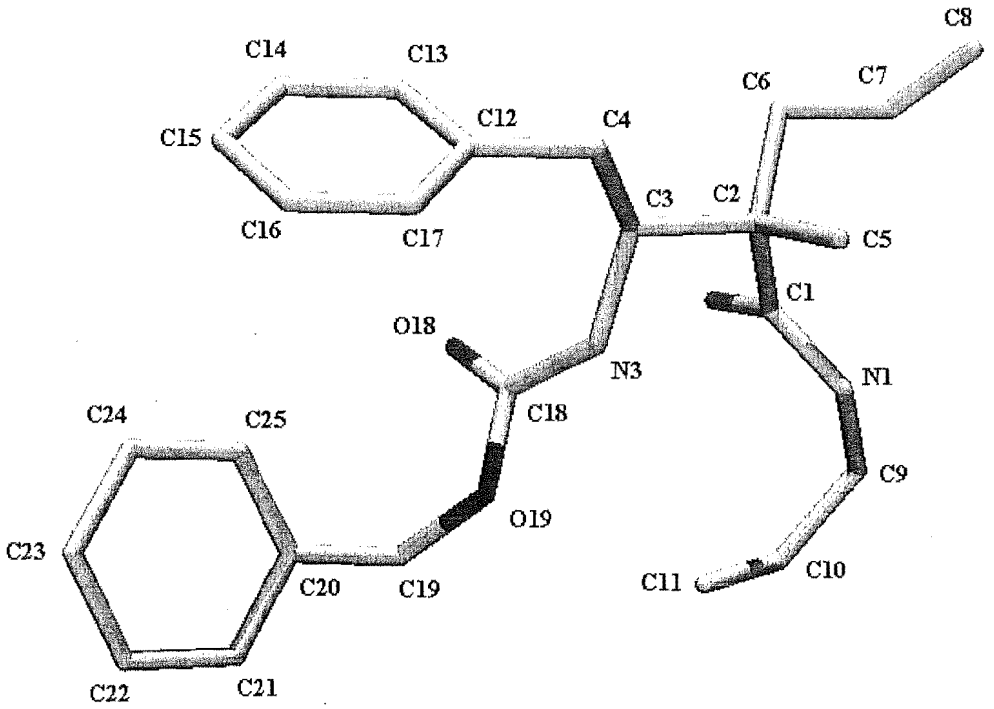
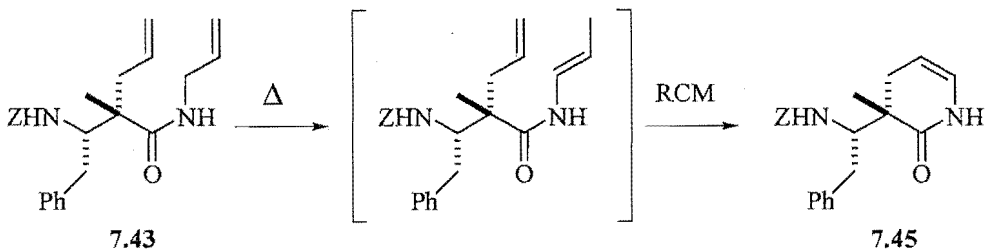


Figure 7.10. Solid-state structure of **7.43**.

Subsequent treatment of diene **7.43** with catalyst **1.42**, in *dry degassed* benzene at 80° C for 4h, gave, after purification by silica chromatography, a single compound in 88% yield. Analysis by ^{1}H NMR and mass spectrometry revealed that RCM of diene **7.43** under these conditions had resulted in a ring-contraction to form the 6-membered ring lactam **7.45**, rather than the anticipated 7-membered lactam **7.44** (Scheme 7.15).



7.43 **7.45**

Scheme 7.15. *Reagents and Conditions*: catalyst **1.42**, benzene, 80° C, 4h, 88%.

Formation of **7.45** had presumably occurred via migration of the amide olefin, as a result of the elevated reaction temperature, in a manner similar to that observed in the formation of the α-amino acid derived lactam **7.26** (Scheme 7.9). However, this ring contraction was not observed during the preparation of the α-unsubstituted β-amino acid derived lactam **7.38** carried out under similar conditions, suggesting that the α-methyl group may promote migration of the amide olefin prior to RCM.

Note also that RCM of **7.43**, to form **7.45**, occurred readily, and in good yield, despite the lack of a substituent on the allyl amide nitrogen, an apparent requirement for ring-closure of equivalent α,α-disubstituted α-amino acid derived compounds (Scheme 7.11). This was also observed during the preparation of the α-free lactam **7.38**, and suggests that dienes derived from β-amino acids are more amenable to RCM than those derived from α-amino acids. This is of particular interest in the synthesis of the α-substituted examples where, in the case of the β-amino acid derived compounds, derivatisation of the lactam nitrogen is not constrained to occur prior to RCM (see Schemes 7.10 and 7.11). This lends itself to a greater degree of flexibility during the synthesis of cyclic lactams of this type.

Key ^{1}H NMR chemical shifts for **7.45**, and associated connectivity correlations from 2D COSY, HSQC and HMBC experiments are shown in Figure 7.11.

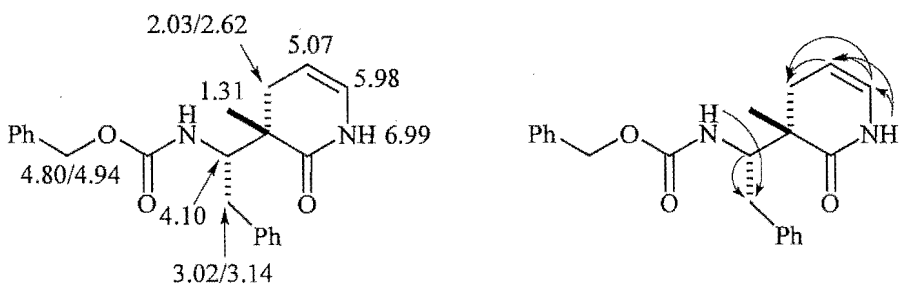
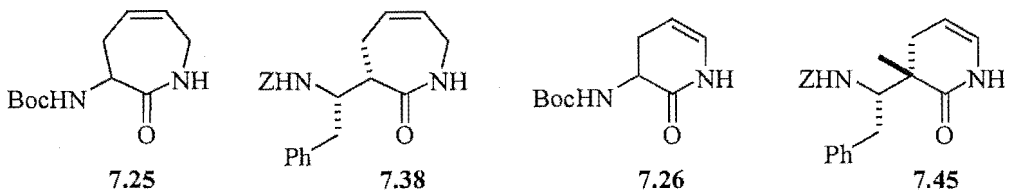


Figure 7.11. Key ^{1}H NMR chemical shifts and connectivity data for **7.45**.

Key observations include correlations between the lactam NH and the adjacent olefin, thereby establishing the connectivity of the ring, and correlations between the benzyloxycarbonylamino NH and the β-amino acid side-chain confirming connectivity exocyclic to the ring. The structure of **7.45** was further supported by mass spectrometry (TOF MS ES+ 365.1866), with the ^{1}H chemical shifts observed for the olefinic protons in **7.45** (δ_N = 5.07 and 5.98ppm) also consistent with those observed for the equivalent protons in the 6-membered α-amino acid derived lactam **7.26** (refer Figure 7.7). In addition, the difference in chemical shift between adjacent olefinic protons in the 6-membered lactams **7.26** and **7.45** corresponds to ~0.9ppm, while the difference between equivalent protons in the 7-membered lactams **7.25** and **7.38** is ~0.1ppm. This difference in chemical shift between equivalent olefinic protons of the 6- and 7-membered lactams provides a key diagnostic tool for distinguishing between the two ring systems. This information is summarised in Figure 7.12 along side associated lactam NH chemical shifts.



7.25 **7.38** **7.26** **7.45**

	7-membered lactams		**6-membered lactams**	
H-5	5.74	5.71	5.16	5.07
H-6	5.68	5.61	6.06	5.98
N-H	6.08	6.48	7.67	6.99

Figure 7.12. Comparison of the chemical shifts of the olefinic and lactam amide protons for the 7-membered lactams **7.25** and **7.38** and the 6-membered cyclic lactams **7.26** and **7.45**.

It is envisaged that formation of the 7-membered ring lactam **7.44** (refer Scheme 7.14) could be achieved by lowering the temperature of reaction during RCM of diene **7.43**, however this was not carried out here. Nevertheless, the preparation of **7.45** represents the first example of α-substituted lactams of this type incorporating a functionalised β-amino acid.

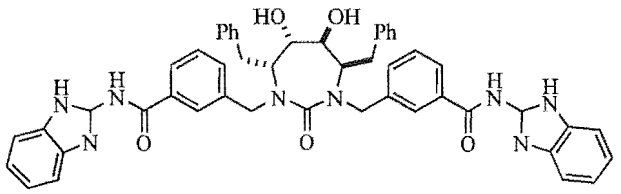
7.4 Conclusion and Future Work

In summary, we have demonstrated the ability to prepare either 6- or 7-membered lactams from an allyl glycine-derived diene **7.24**, using RCM chemistry. RCM of diene **7.24** at 85° C resulted in exclusive formation of the 7-membered lactam **7.25**, while RCM at 100° C gave a 1:1 mixture by ^{1}H NMR of **7.25** and the 6-membered lactam analogue **7.26**. This is the first observed isomerization of this type during RCM.

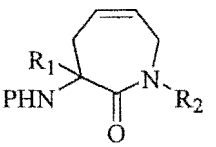
Suitable methodology was also developed for the synthesis of dienes of type **7.27**, substituted at the α-position, that were envisaged to undergo RCM to form 7-membered lactams of type **7.28**. Priestley and Decicco subsequently demonstrated that a substituent was required on the allyl amide nitrogen before RCM will occur.

Methodology has also been developed for the preparation of equivalent 6- and 7-membered lactams derived from a common β-amino acid precursor, with these being an important addition to this class of compound. Dienes of type **7.37** and **7.43**, that are either unsubstituted, or substituted at the α-position, have been shown to undergo RCM to form the 7-membered lactam **7.38**, and the 6-membered lactam **7.45**, respectively. Initial efforts focussed on lactams derived from Cbz-protected methyl 4-phenyl-3-aminobutanoate **7.34**. Alkylation on the lactam nitrogen of **7.38**, to give the dipeptide **7.39**, further illustrated the ability of these compounds to be incorporated into peptide sequences. Formation of the 6-membered lactam **7.45** is consistent with olefinic migration of the terminal alkene of **7.43**, brought about by the elevated temperature of the reaction conditions (85° C). It is envisaged that preparation of the

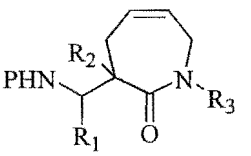
equivalent 7-membered lactam **7.44** can be achieved by lowering the temperature at which this reaction is carried out, in a manner similar to that seen for the allylglycine derived **7.25**. Future work in this area is envisaged to involve the olefinic derivatisation of compounds of this type, with subsequent monomers finding incorporation into larger structures for use in the development of novel peptidomimetics. This methodology is amenable to the use of a variety of starting amino acids with hydroxylated derivatives of this type also offering potential as HIV-1 protease inhibitor scaffolds similar in structure to the potent cyclic urea SD-146.



SD-146



7.21



7.22

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CHAPTER EIGHT

EXPERIMENTAL

8.1 General Methods and Experimental Procedures

Melting Points

All melting points were obtained on an Electrothermal apparatus and are uncorrected.

Nuclear Magnetic Resonance

Proton NMR and nOe spectra were obtained on a Varian Inova spectrometer, operating at 500 MHz. Carbon NMR spectra were obtained on a Varian Unity 300 spectrometer, operating at 75 MHz, with a delay (D_1) of 1 s. All spectra were obtained at 23 °C unless specified otherwise. Chemical shifts are reported in parts per million (ppm) on the δ scale. Solvents used for NMR analysis (reference peak listed) included: $CDCl_3$ ($\mathbf{C}HCl_3$ at δ_H 7.25ppm, $\mathbf{C}DCl_3$ at δ_C 77.0ppm); CD_3OD (CHD_2OD at δ_H 3.30ppm, $\mathbf{C}D_3OD$ at δ_C 49.3ppm). Two-dimensional NMR experiments included COSY, HSQC, HMBC, and CIGAR , and were obtained on the Varian Inova spectrometer operating at 500 MHz. The HSQC, HMBC and CIGAR experiments were all obtained with a delay (D_1) of 1s.

Infrared Spectroscopy

Infrared spectra were obtained using a Shimadzu 8201PC series FTIR interfaced with an Intel 486 PC operating Shimadzu's HyperIR software. Spectra were obtained in either $CHCl_3$ (solution phase) or solid KBr (diffuse reflectance).

Small Molecule Mass Spectrometry

Electron impact (EI) mass spectra were detected on a Kratos MS80 RFA mass spectrometer operating at 4000 V (accelerating potential) and 70 eV (ionization energy). The source temperature was 200-250 °C Electrospray ionization (ESI) mass spectra were detected on a Micromass LCT TOF mass spectrometer, with a probe voltage of 3200 V, temperature of 150 °C and a source temperature of 80 °C. Direct ionization used 10μL of a 10μg mL^{-1} solution, using a carrier solvent of 50% acetonitrile/H_2O at a flow rate of 20μL min^{-1}. Ionization was assisted by the addition of 0.5% formic acid.

Microanalysis

Microanalysis was performed at the University of Otago Microanalytical Laboratory. All reported values are within ±0.4% of the calculated value.

Optical Rotations

Optical rotations were measured on a Perkin Elmer polarimeter Model 341, with a 10mm path length. The $[\alpha]_D$ values are given in units of deg cm^2 g^{-1}, with the concentrations given in $10^{-1}gcm^{-3}$.

Reagents, Solvents and Laboratory Methodology

Oven-dried glassware was used in all reactions carried out under an inert atmosphere (either dry nitrogen or argon). All starting materials and reagents were obtained commercially unless otherwise stated. Removal of solvents "under reduced pressure" refers to the process of bulk solvent removal by rotary evaporation (low vacuum pump) followed by application of high vacuum (oil pump) for a minimum of 30min. Analytical thin layer chromatography (TLC) was performed on plastic-backed Merck Kieselgel $KG60F_{254}$ silica plates, and visualized using short wave ultraviolet light, $KMnO_4$ or phosphomolybdate dip. Flash chromatography was performed using 230-400 mesh Merck Silica Gel 60 following established guidelines under positive pressure.[1] Radial chromatography was performed using a Harrison Chromatotron using 1mm, 2mm and 4mm silica gel plates under positive pressure. THF and diethyl ether were distilled from sodium benzophenone ketyl under an inert atmosphere immediately prior to use. Dichloromethane, 1,2-dichloroethane, benzene and toluene were distilled from calcium hydride under an inert atmosphere. Petroleum ether refers to the fraction collected between 60-70 °C. Ethyl acetate and petroleum ether were distilled from calcium hydride prior to their use in chromatography. DMF was dried by placing over 4Å molecular sieves, applying a high vacuum for 15 min, then flushing briefly with an inert atmosphere. This process was repeated twice more over 24 h, after which the DMF was stored over 4Å molecular sieves under an inert atmosphere. All other reagents and solvents were purified prior to use according to literature procedures.[2]

General Procedure A: Preparation of Phenyl-5-Oxazolidinones

Amino acid (1 equiv) was dissolved in 1M aq. NaOH (1 equiv) with gentle warming. The solution was evaporated under reduced pressure and the resulting white solid placed under high vacuum. The white solid was dissolved in 1,2-dichloroethane (~0.3 M) to give a suspension. Benzaldehyde (1.5 equiv) was then added and the mixture refluxed with azeotropic removal of water using a Dean-Stark apparatus, for 24 h. The reaction mixture was cooled to –20 °C under nitrogen, and benzoyl chloride (1 equiv) added. The mixture was stirred at –20 °C for 4 h, then at 0 °C for 3 days. Upon warming to rt the mixture was washed with 5% aq. $NaHCO_3$, 5% aq. $KHSO_4$, and H_2O, dried ($MgSO_4$, and the solvent removed under reduced pressure. Recrystallisation of the residue from MeOH gave the desired 5-oxazolidinone.

Modified General Procedure A.[3]

Amino acid (1 equiv) was dissolved in 1 M aq. NaOH (1 equiv) with gentle warming. The solvent was evaporated under reduced pressure and the resulting white solid placed under high vacuum. The white solid was then dissolved in *dichloromethane* (~0.3 M) to give a suspension. Benzaldehyde (1.5 equiv) was then added and the mixture refluxed, with azeotropic removal of water using a Dean-Stark apparatus, for 5-8 h. The reaction mixture was cooled to 0 °C under nitrogen, and benzoyl chloride (1 equiv) added. The mixture was stirred at 0 °C for 4 h, then at rt for 16 h. The solution was washed with 5% aq. $NaHCO_3$, 5% aq. $KHSO_4$, and H_2O, dried ($MgSO_4$), and the solvent removed under reduced pressure. Recrystallisation of the residue from MeOH gave the desired 5-oxazolidinone.

General Procedure B: Hydrolysis of Oxazolidinones to Carboxylic Acids

Sodium hydroxide (2 equiv) was added to oxazolidinone (1 equiv) dissolved in MeOH (~0.1 M) and the mixture refluxed for 1 h. The solution was cooled and concentrated under reduced pressure. The residue was taken up in water, acidified to pH 1 with 10% HCl and extracted with ether (3x10mL)). The combined ether extracts were washed with NaCl(10mL), dried ($MgSO_4$) and the solvent removed under reduced pressure to give the carboxylic acid.

General Procedure C: Esterification of Carboxylic Acids with CH_2N_2

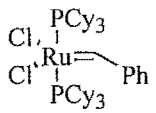
Ethereal diazomethane was added to a 0 °C solution of carboxylic acid in ether (~0.1 M), until the bright yellow colour persisted over an extended period. The mixture was then stirred at rt for 2 h, whereupon the reaction was quenched with the addition of a few drops of acetic acid. The solvent was removed to give the methyl ester

.

General Procedure D: Allylation on Nitrogen

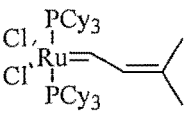
A solution of methyl ester (1 equiv) dissolved in dry dimethylformamide (~0.1 M) was cooled to 0 °C under nitrogen. Allyl bromide (3 equiv) was added, followed by slow addition of NaH (60% in oil, 3 equiv). Bubbling was observed as H_2 was released. The solution was stirred at 0°C for 1.5 h and at rt for 30 min. The mixture was quenched with sat. aq. NH_4Cl and extracted with ethyl acetate (3x10mL)). The ethyl acetate extracts were combined and washed with water (2x10mL), brine(10mL), dried ($MgSO_4$), and the solvent removed under reduced pressure. The residue was purified by silica chromatography to give the *N*-allylated product

General Procedure E: Ring-Closing Metathesis



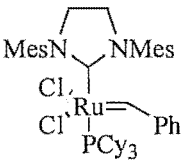
1.40

Bis(tricyclohexylphosphine) benzylidene ruthenium(IV) dichloride



1.41

Bis(tricyclohexylphosphine)-3-methyl-2-butenylidene ruthenium(IV) dichloride



1.42

Tricyclohexylphosphine[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazole-2-ylidene benzylidene]ruthenium(IV) dichloride

The ruthenium catalyst (either **1.40**, **1.41**, or **1.42**), dissolved in *dry degassed*[a] benzene or CH_2Cl_2, was added to a solution of diene (1 equiv) dissolved in *dry degassed* benzene or CH_2Cl_2 (~0.1mmol) under nitrogen. The solution was stirred at rt, or refluxed, for 2 h or a specified time (see experimental for details). Purification by radial chromatography gave the desired olefinic product.

General Procedure F: Hydrogenation of Olefins

10% Palladium-on-carbon (20% w/w) was added to a solution of olefin (1 equiv) dissolved in dry MeOH (~0.05 M), and the solution stirred vigorously under hydrogen for 12 h. The mixture was then filtered through a small bed of Celite^{TM}, washing with MeOH, and the solvent removed under reduced pressure to give the product

General Procedure G: Methyl Ester Hydrolysis

Sodium hydroxide (2 equiv of 1M aq.) was added to a solution of methyl ester (1 equiv) dissolved in MeOH (~0.05 M), and the solution refluxed for 4 h. The MeOH was removed under reduced pressure and water added to make up to a workable amount. The solution was acidified to pH 2 with 10% HCl and extracted with ethyl acetate (3x10mL). The combined ethyl acetate extracts were dried over $MgSO_4$ and the solvent removed under reduced pressure to give the free acid.

General Procedure H: Preparation of *tert*-Butyl-5-Oxazolidinones

Amino acid (1 equiv) was dissolved in 1 M aq. NaOH (1 equiv) with gentle warming. The solvent was evaporated under reduced pressure and the resulting white solid placed under high vacuum. The white solid was dissolved in *pentane* (~0.3 M) to give a suspension, and trimethylacetaldehyde (pivaldehyde) (1.5 equiv) added. The mixture was then refluxed, with azeotropic removal of water using a Dean-Stark apparatus, for 5-8 h. The solution was cooled to 0 °C under nitrogen, benzoyl chloride (1 equiv) added, and the mixture stirred at 0 °C for 48 h. The mixture was washed with 5% aq. $NaHCO_3$, 5% aq. $KHSO_4$, and H_2O, dried ($MgSO_4$), and the solvent removed under reduced pressure. Recrystallisation from MeOH gave the 5-oxazolidinone.

[a] Degassed solvents were obtained using a "freeze-thaw" method in which the solvent was frozen solid under an inert atmosphere, and subsequently thawed under high vacuum. This process was repeated several times until the solvent was sufficiently free of oxygen.

General Procedure I: Conversion of Methionine Side Chain to a Vinyl Group via Oxidative Elimination

a) Hydrogen peroxide (50% w/w solution, 1.4 equiv) was added a solution of the methionine derivative (1equiv) dissolved in acetic acid (~0.5 M), and the mixture stirred at rt for 4 h. CH_2Cl_2 (20mL) was added to the solution and the solution carefully neutralised with sat. aq. Na_2CO_3 (vigorous bubbling was observed). The organic phase was then washed with water (10mL), dried ($MgSO_4$), and the solvent removed under reduced pressure, to give the intermediate sulfoxide.

b) The sulfoxide was then dissolved in degassed *m*-xylene and sealed, under vacuum, in a glass tube. The tube was then heated at 200 °C in a Kugelröhr for 16 h. The solvent was then removed to give a brown solid, with purification by silica chromatography giving the desired vinyl derivative.

General Procedure J: Synthesis of β-Amino Acid Methyl Ester Derivatives from α-Amino Acids[4]

a) Triethylamine (1 equiv) and ethyl chloroformate (1 equiv) were added under argon to a –15 °C solution of the *N*-protected amino acid dissolved in THF (0.2 M). After 15 min the suspension was allowed to warm to 0 °C, whereupon ethereal diazomethane was added until the intensive yellow colour persisted over a longer period. (The colour of the diazoketone is light yellow.) The mixture was allowed to warm to rt and then stirred for 3h. Excess diazomethane was destroyed by vigorous stirring, or by the addition of a few drops of AcOH. After aqueous workup by extraction with satd. $NaHCO_3$ (10mL), NH_4Cl (10mL), and NaCl (10mL) solutions, the organic solution was separated, dried ($MgSO_4$), and the solvents evaporated under reduced pressure. Chromatography of the residue on silica gel, or recrystallisation, afforded the pure diazoketone.

b) The diazoketone was dissolved in dry MeOH (~0.25 M) under argon at –25 °C with the exclusion of light. Silver benzoate (0.11 equiv), dissolved in Et_3N (2.9 equiv), was added and the mixture was allowed to warm to rt within 3 h. The solvent was removed under reduced pressure and the residue was taken up in ethyl acetate. After workup by extraction with satd. $NaHCO_3$ (10mL), NH_4Cl (10mL), and NaCl (10mL) solutions, drying ($MgSO_4$),

the solvents were removed under reduced pressure. Purification by silica chromatography, or recrystallisation, gave the homoamino acid methyl ester.

General Procedure K: Synthesis of α-Substituted β-Amino Acid Derivatives

Anhydrous LiCl (3 equiv) was suspended in THF (*Z*-protected β-amino acid methyl ester should be 0.2 M) and cooled to –78 °C. LDA (2.2 equiv) was added and the solution stirred at –78 °C for 10 min. The Z-protected β-amino acid methyl ester (1 equiv) was then added and the mixture was stirred at –78 °C for 1 h. The electrophile (4 equiv) was added slowly and the mixture was stirred at –78 °C for 2 h and then allowed to warm to rt over 16 h. Hydrolysis was carried out by addition of NH_4Cl and after workup, by extraction with satd. $NaHCO_3$ (10mL), NH_4Cl (10mL), and NaCl (10mL) solutions, and drying ($MgSO_4$), the solvents were removed under reduced pressure. Purification by silica gel chromatography gave the desired product.

General Procedure L: Hydrogenation of *N*-Cbz-protected Olefins, Reprotection with Cbz

10% Palladium-on-carbon (20% w/w) was added to a solution of olefin (1 equiv) dissolved in MeOH (~0.05 M) and the solution stirred vigorously under hydrogen for 12 h. The mixture was then filtered through Celite™ and the solvent removed under reduced pressure. The residue was then taken up in CH_2Cl_2 (~0.1 M) and diisopropylethylamine (2.8 equiv) was added, followed by benzylchloroformate (1.6 equiv) and dimethylaminopyridine (0.3 equiv), and the solution stirred overnight at rt. The solvent was removed under reduced pressure and the residue taken up in ethyl acetate and successively washed with sat. aq. $NaHCO_3$ (10mL), NaCl (10mL), water (10mL), dried ($MgSO_4$) and the solvent removed to give the product.

Modified General Procedure L: Hydrogenation of *N*-Cbz-protected Olefins, Reprotection with Boc

10% Palladium-on-carbon (20% w/w) was added to a solution of olefin (1equiv) dissolved in dry MeOH (~0.05 M), and the solution stirred vigorously under hydrogen for 12 h. The mixture was then filtered through a small bed of Celite™, washing with MeOH, and the

solvent volume reduced to approximately 2mL. $NaHCO_3$ (1.5equiv) and di-*tert*-butyl-dicarbonate (1.5 equiv) were then added and the solution stirred at rt for 3 h. The solvent was removed and the residue purified by silica chromatography to give the product.

General Procedure M: Curtius Rearrangement – Acids to Amides

Et_3N (1.2 equiv) and $ClCO_2Et$ (1.1 equiv) were added to a 0 °C solution of the acid (1 equiv) dissolved in dry acetone (~0.1 M) under argon, and the mixture stirred at 0 °C for 1 h. NaN_3 (2.5 equiv) in H_2O, was then added and the mixture stirred at 0 °C for a further 1h. The solvent was evaporated below rt and the residue extracted with toluene (3x10mL). The combined organic extracts were dried over $MgSO_4$ and heated carefully using a distillation condenser. The volume was reduced to ~10mL over a 1 h period. Alcohol (^tBuOH or BnOH, 3 equiv) was added and the distillation condenser replaced with a reflux condenser. The reaction was gently refluxed for 12 h. The solvent was removed under reduced pressure and the residue diluted with CH_2Cl_2 (20mL), washed with 3 M aqueous HCl (10mL), water (10mL), brine (10mL), dried ($MgSO_4$), and the solvent removed under reduced pressure to give the product.

Modified General Procedure M:

Et_3N (1.2 equiv) and diphenylphosphorylazide (DPPA) (1 equiv) was added to a solution of the acid (1 equiv) dissolved in toluene (~0.1 M) under argon. The solution was stirred at rt for 30min before being slowly heated to reflux (N_2 bubbling was observed at 70-80 °C). Once bubbling has ceased (~3 h), the reaction was cooled to 50 °C, alcohol (^tBuOH or BnOH) (3 equiv) added, and the solution refluxed for a further 3 h. Upon cooling to rt, the reaction was quenched with sat. aq. $NaHCO_3$ (10mL), and the mixture extracted with ether (3x10mL)). The combined ether extracts were dried ($MgSO_4$) and the solvent removed under reduced pressure. Chromatography (EA/PE) gave the product .

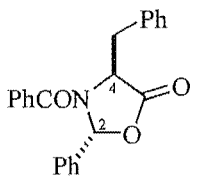
General Procedure N: Coupling of Carboxylic Acids with Allylamine

EDCI (1.3 equiv), HOBt (1.5 equiv), allylamine (1.5 equiv) and diisopropylethylamine (1.1 equiv) were added to a solution of the acid (1 equiv) dissolved in CH_2Cl_2 (~0.1 M) under argon. The mixture was stirred at rt under argon for 16 h. The solution was diluted

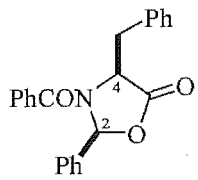
with CH_2Cl_2 (10mL), washed successively with 10% aqueous HCl (10mL), water (10mL), dried over Na_2SO_4, and the solvent removed to give the product.

8.2 Experimental Work Described in Chapter Two

Preparation of (4*S*,2*R*)-3-Benzoyl-4-benzyl-2-phenyl-oxazolidin-5-one (+)-2.4, and (4*S*,2*S*)-3-Benzoyl-4-benzyl-2-phenyl-oxazolidin-5-one 2.10.



(+)-2.4



2.10

Method A: (*S*)-Phenylalanine (10g, 60.6mmol, 1 equiv) was reacted with 1M aq. NaOH (60.6mL, 60.6mmol, 1 equiv), and condensed with benzaldehyde (9.24mL, 90.9mmol, 1.5 equiv) according to General Procedure A. Cyclisation with benzoyl chloride (7.035mL, 60.6mmol, 1 equiv) gave a crude mixture containing a 4:1 ratio, by ^{1}H NMR, of **2.4** and **2.10**. Recrystallisation from MeOH gave the *trans*-oxazolidinone (+)-**2.4** (7.327g, 34%) as white needles

Method B:[3] (*S*)-Phenylalanine (8g, 48.5mmol, 1 equiv) was treated with 1M aq. NaOH (48.5mL, 48.5mmol, 1 equiv) and condensed with benzaldehyde (7.4mL, 72.7mmol, 1.5 equiv) according to Modified General Procedure A. Cyclisation with benzoyl chloride (5.64mL, 48.5mmol, 1 equiv) gave a crude mixture containing a 4:1 ratio of **2.4** and **2.10**. Recrystallisation of the residue from MeOH gave the *trans*-oxazolidinone (+)-**2.4** (10.206g, 59%) as white needles

Data for (+)-2.4:

mp 182-184° C (lit.[3] 184.3° C),

^{1}H NMR ($CDCl_3$) δ 3.43 (brs, 1H, C**H**$_a$Ph), 3.79 (brs, 1H, C**H**$_b$Ph, 5.22 (s, 1H, **H4**), 5.82 (s, 1H, **H2**), 7.06-7.41 (m, 15H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 34.7, 57.6, 91.2, 126.6, 127.7, 128.5, 128.8, 129.8, 129.8, 130.7, 135.2, 136.1, 169.2, 171.2.

FTIR (KBr) 1802, 1655cm^{-1}

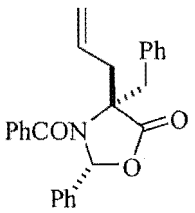
HMRS 357.1366 (M^+). $C_{23}H_{19}NO_3$ requires 357.1365.

$[\alpha]_D$ = +267°, c=1.0 $CHCl_3$ (lit.[3] +385.2°, c=1.0, $CHCl_3$).

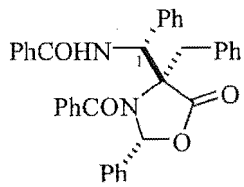
Selected data for 2.10 (from crude):

^{1}H NMR ($CDCl_3$) δ 4.69 (m, 1H, **H4**), 4.90 (brs, 1H, **H2**).

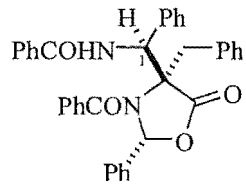
Preparation of (4*R*,2*R*)-4-Allyl-benzoyl-4-benzyl-2-phenyl-oxazolidin-5-one (-)-2.5, (2*R*,4*S*,1'*R*)-3-Benzoyl-4-[benzoylamino(phenyl)methyl]-4-benzyl-2-phenyl-1,3-oxazolidin-5-one 2.11, and its C-1' epimer 2.18.



(-)-2.5



2.11



2.18

LiHMDS (6.501mL of 1M solution in THF/hexanes, 6.5mmol, 1.1 equiv) was added, under argon, to a solution of the oxazolidinone (+)-**2.4** (2.11g, 5.91mmol, 1 equiv) in dry THF (15mL) at -78°C. The solution was stirred at -78° C for 7min, whereupon allyl bromide (0.768mL, 8.87mmol, 1.5 equiv) was added and the mixture stirred at -78°C for 2 h, before being allowed to warm to rt overnight. Sat. aq. NH_4Cl solution (20mL) was added and the solution extracted with ether (3x20mL). The ether extracts were combined, dried (Na_2SO_4) and the solvent removed to give the product as a white solid. Recrystallisation from ether gave (-)-**2.5** (2.342g, 93%) as white needles. Purification of the remaining residue by radial chromatography (EA/PE 1:3) gave the self addition product **2.11** (96mg, 6%),[5] as a white solid.

Treatment of (+)-**2.4** with LiHMDS for extended periods (20-60min) before the addition of allyl bromide resulted in crude mixtures of (-)-**2.5** and **2.11** being observed by ^{1}H NMR. :

Treatment of (+)-**2.4** (200mg, 0.56mmol, 1 equiv) with LiHMDS (0.72mL of 1M solution in THF/hexanes, 0.72mmol, 1.2 equiv) in the absence of allyl bromide gave a crude mixture containing **2.11** by ^{1}H NMR and <5% of a second diastereoisomer tentatively assigned as the C-1' epimer **2.18**. Purification by radial chromatography (EA/PE 1:3) gave **2.11** (84mg, 55%) as a white solid.

Data for (-)-**2.5**: An analytical sample was obtained by the diffusion of petroleum ether into a solution of (-)-**2.5** dissolved in ethyl acetate. Crystals suitable for X-ray crystallography were obtained (see Appendix for data).

mp 141-143° C.

^{1}H NMR 500MHz ($CDCl_3$) δ 2.82 (dd *J*=13.7 and 5.4Hz, 1H, C**H**$_a$CH=CH$_2$), 3.45 (d *J*=13.7Hz, 1H, C**H**$_a$Ph), 3.59 (dd *J*=13.7 and 10.1Hz, 1H, C**H**$_b$CH=CH$_2$), 4.09 (d *J*=13.7Hz, 1H, PhC**H**$_b$), 5.50 (m, 4H, CH=C**H**$_2$ and Ph**H**), 5.98 (m, 1H, C**H**=CH$_2$), 6.03 (s, 1H, **H2**), 6.66 (t *J*=7.8Hz, 2H, Ph**H**), 6.77 (d *J*=7.3Hz, 2H, Ph**H**), 6.95 (t *J*=7.3Hz, 1H, Ph**H**), 7.02 (t *J*=7.5Hz, 2H, Ph**H**), 7.14 (t *J*=7.5Hz, 1H, Ph**H**), 7.38-7.45 (m, 5H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 39.7, 41.6, 69.8, 91.0, 122.2, 125.2, 127.4, 127.6, 127.8, 128.2, 129.0, 129.1, 129.1, 130.8, 131.3, 135.0, 136.2, 136.6, 169.9, 173.3.

FTIR (KBr) 3026, 1792, 1653cm^{-1}

HRMS (EI) 397.1672 (M^+). $C_{26}H_{23}NO_3$ requires 397.1678.

$[\alpha]_D$ = -2.9° c=1.0 $CHCl_3$

Data for **2.11**:

^{1}H NMR ($CDCl_3$) δ 3.61 (d *J*=13.9 Hz, 1H, C**H**$_a$Ph), 4.46 (d *J*=13.9 Hz, 1H, C**H**$_b$Ph), 4.72 (s, 1H, **H2**), 5.25 (d *J*=7.3 Hz, 2H, Ph**H**), 6.21 (d *J*=8.8 Hz, 1H, C**H**NH), 6.56 (t *J*=7.8 Hz, 2H, Ph**H**), 6.67 (d *J*=6.8 Hz, 2H, Ph**H**), 6.87 (t *J*=7.4 Hz, 1H, Ph**H**), 6.98 (t *J*=7.8 Hz, 2H, Ph**H**), 7.10 (t J=7.8 Hz, 1H, Ph**H**), 7.41-7.62 (m, 13H, Ph**H**), 8.08 (d J=6.8 Hz, 2H, Ph**H**), 9.52 (d J=8.8 Hz, 1H, N**H**).

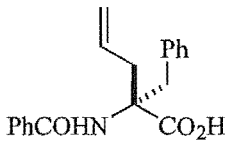
^{13}C NMR ($CDCl_3$) δ 38.1, 60.8, 74.7, 90.8, 124.7, 127.3, 127.5, 127.6, 127.8, 127.96, 128.3, 128.7, 129.0, 129.2, 129.3, 129.4, 131.1, 131.8, 133.6, 133.8, 135.2, 135.7, 137.6, 166.2, 171.5, 172.7.

FTIR (KBr) 3350, 1791, 1667cm^{-1}.

HRMS (ES) 567.2276 (M^++H). $C_{37}H_{31}N_2O_4$ requires 567.2284.

Selected data for C-1' epimer **2.18** (from crude mixture): ^{1}H NMR ($CDCl_3$) δ 3.90 (d, *J*=13.6Hz, 1H, C**H**$_a$Ph), 4.41 (dd *J*=13.6Hz, 1H, C**H**$_b$Ph), 4.92 (s, 1H, **H-2**), 5.31 (dd *J*=7.2 and 1.3Hz, 2H, Ph**H**).

Preparation of (2*R*)-2-Benzoylamino-2-benzyl-pent-4-enoic acid 2.6.



Oxazolidinone (-)-**2.5** (2.9g, 7.3mmol) was dissolved in MeOH (60mL) and hydrolysed with NaOH (585mg, 14.61mmol, 2 equiv) according to General Procedure B, to give acid **2.6** (2.254g, 100%) as a white solid.

mp 178-180° C.

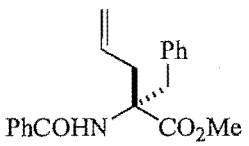
^{1}H NMR (300MHz $CDCl_3$) δ 2.81 (dd *J*=7.0 and 14.0Hz, 1H, C**H**$_a$CH=CH$_2$), 3.32 (d *J*=13.7Hz, 1H, C**H**$_a$Ph), 3.57 (dd *J*=7.8 and 13.7Hz, 1H, C**H**$_b$CH=CH$_2$), 3.93 (d *J*=13.2Hz, 1H, CH$_b$Ph), 5.16 (m, 2H, CH=C**H**$_2$), 5.72 (m, 1H, C**H**=CH$_2$), 6.99 (s, 1H, N**H**), 7.18 (m, 5H, Ph**H**), 7.42 (t *J*=7.8Hz, 2H, Ph**H**), 7.52 (t *J*=7.3Hz, 1H, Ph**H**), 7.67 (d *J*=7.3Hz, 2H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 39.4, 40.1, 66.2, 119.7, 126.9, 127.0, 128.3, 128.7, 129.8, 131.8, 131.8, 134.7, 135.9, 168.0, 176.3.

FTIR (KBr) 3385, 1728, 1618cm^{-1}.

HRMS (EI) 309.1360 (M^+). $C_{19}H_{19}NO_3$ requires 309.1365.

Preparation of (2*R*)-2-Benzoylamino-2-benzyl-pent-4-enoic acid methyl ester (-)-2.7.



Acid **2.6** (1.790g, 5.79mmol) was dissolved in ether (50mL) and esterified with ethereal diazomethane according to General Procedure C, to give methyl ester (-)-**2.7** (1.864g, 99%) as a colourless oil.

^{1}H NMR (500 MHz $CDCl_3$) δ 2.72 (dd *J*=7.3Hz, 1H, C**H**$_a$CH=CH$_2$), 3.21 (d *J*=13.7Hz, 1H, PhC**H**$_a$), 3.60 (dd *J*=7.3Hz, 1H, C**H**$_b$CH=CH$_2$), 3.83 (s, 3H, O**Me**), 3.95 (d *J*=13.7Hz, 1H, PhC**H**$_b$), 5.10 (m, 2H, CH=C**H**$_2$), 5.65 (m, 1H, C**H**=CH$_2$), 6.93 (br s, 1H, N**H**), 7.19 (m, 2H, Ph**H**), 7.41 (m, 3H, Ph**H**), 7.41 (t *J*=7.3Hz, 2H, Ph**H**), 7.49 (t *J*=7.3Hz, Ph**H**), 7.68 (m, 2H, Ph**H**).

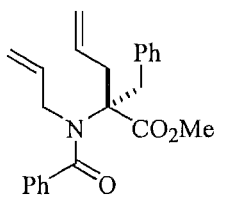
^{13}C NMR ($CDCl_3$) δ 39.3, 40.1, 52.6, 66.2, 119.1, 126.6, 126.8, 128.1, 128.4, 129.5, 131.3, 132.1, 135.1, 136.1, 166.7, 173.2.

FTIR (KBr) 3414, 2953, 1738, 1666, 1603, 1580, 1516, 1487, 1448cm^{-1}

HMRS (EI) 323.1525 (M^+). $C_{20}H_{21}NO_3$ requires 323.1521.

$[\alpha]_D$ = –56.8°, c-1.0 $CHCl_3$.

Preparation of (2*R*)-2-(Allyl-benzoyl-amino)-2-benzyl-pent-4-enoic acid methyl ester (+)-2.8.



Methyl ester (-)-**2.7** (4g, 12.4mmol, 1 equiv) was dissolved in DMF (120mL) and reacted with allyl bromide (3.215mL, 37.2mmol, 3 equiv) and NaH (1.486g of 60% in oil, 37.2mmol, 3 equiv) according to General Procedure D. Purification by column chromatography (EA/PE 1:3) gave 920mg (23%) of starting material (-)-**2.7**. Further elution gave.diene (+)-**2.8** (1.343g, 30%) as a white solid.

mp 92-93°C.

^{1}H NMR ($CDCl_3$) δ 2.73 (m, 2H, NC**H**$_2$CH=), 3.09 (m, 1H, CC**H**$_a$CH=), 3.22 (d *J*=13.7Hz, 1H, PhC**H**$_a$), 3.63 (m, 1H, CC**H**$_b$CH=), 3.69 (d *J*=13.7Hz, 1H, PhC**H**$_b$), 3.75 (s, 3H, O**Me**), 5.08-5.34 (m, 4H, 2xCH=C**H**$_2$), 5.54 (m, 1H, NCH$_2$C**H**=CH$_2$), 5.87 (m, 1H, CCH$_2$C**H**=CH$_2$), 7.21-7.43 (10H, Ph**H**).

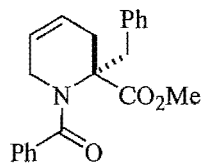
^{13}C NMR ($CDCl_3$) δ 36.1, 36.6, 49.4, 52.0, 67.9, 116.6, 119.9, 126.2, 126.9, 128.2, 128.3, 129.4, 130.7, 132.2, 136.4, 136.5, 136.8, 172.7, 172.7

FTIR (KBr) 3423, 2924, 1732, 1628cm^{-1}

HRMS (EI) 362.1758 (M^+-H). $C_{23}H_{24}NO_3$ requires 362.1756.

$[\alpha]_D$ = +64.3°, c=1.0 $CHCl_3$.

Preparation of (2*R*)-1-Benzoyl-2-benzyl-1,2,3,6-tetrahydro-piperidine-2-carboxylic acid methyl ester (+)-2.9.



Catalyst **1.40** (114mg, 0.14mmol, 5mol%), dissolved in *dry degassed* CH_2Cl_2 (3mL), was added to a solution of diene (+)-**2.8** (1g, 2.75mmol, 1 equiv) dissolved in *dry degassed* CH_2Cl_2 (25mL) under nitrogen, according to General Procedure E. The mixture was then stirred at rt for 2h. Purification by radial chromatography (EA/PE 1:3) gave (+)-**2.9** (867mg, 94%) as a white solid. An analytical sample was obtained by the diffusion of petroleum ether into a solution of (+)-**2.9** dissolved in ethyl acetate. Crystals suitable for X-ray crystallography were obtained (see Appendix for data).

^{1}H NMR 500MHz ($CDCl_3$) δ 2.43 (m, 1H, CC**H**$_a$CH=), 2.78 (m, 1H, CC**H**$_b$CH=), 3.18 (d *J*=13.2Hz, 1H, PhC**H**$_a$), 3.62 (d *J*=13.2Hz, 1H, PhC**H**$_b$), 3.68 (s, 3H, O**Me**), 3.80 (m, 1H, NC**H**$_a$CH=), 4.10 (m, 1H, NC**H**$_b$CH=), 5.67 (m, 1H, CCH_2C**H**=), 5.88 (m, 1H, NCH_2C**H**=), 7.15-7.52 (m, 10H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 28.5 (CCH_2CH=), 38.9 (**C**H_2Ph), 46.8 (NCH_2CH=) , 51.9 (OCH_3), 62.7 (**C**CO_2Me), 124.6 (CCH_2**C**H=), 125.3 (NCH_2**C**H=), 127.0, 127.9, 128.1, 128.4, 130.5, 130.5, 135.7, 135.8, 172.4, 172.6.

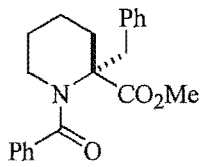
FTIR (KBr) 2947, 1742, 1634cm^{-1}.

HRMS (EI) 335.1520 (M^+). $C_{21}H_{21}NO_3$ requires 335.1521.

Micro. Calcd for $C_{21}H_{21}NO_3$, C, 75.19; H, 6.31; N, 4.18; Found: C, 74.94; H, 6.23; N, 4.19.

$[\alpha]_D$= +38.2°, c=1.0 $CHCl_3$.

Preparation of (2*R*)-1-Benzoyl-2-benzyl-piperidine-2-carboxylic acid methyl ester (+)-2.12.



Olefin (+)-**2.9** (23mg, 0.07mmol) was dissolved in dry MeOH (1.5mL) and reacted with 10% palladium-on-carbon (4.5mg, 20% w/w), under a hydrogen atmosphere according to General Procedure F, to give (+)-**2.12** (21mg, 92%) as a white solid. An analytical sample was obtained by the diffusion of petroleum ether into a solution of (+)-**2.12** dissolved in ethyl acetate. Crystals suitable for X-ray crystallography were obtained (see Appendix for data).

^{1}H NMR ($CDCl_3$) δ 1.35 (m, 1H, C**H**), 1.61 (m, 2H, C**H**$_2$), 1.75 (m, 2H, CC**H**$_a$ and C**H**), 2.15 (m, 1H, CC**H**$_b$), 2.34 (m, 1H, NC**H**$_a$), 3.05 (d *J*=13.7Hz, 1H, PhC**H**$_a$), 3.29 (m, 1H, NC**H**$_b$), 3.81, (s, 3H, O**Me**), 4.05 (d *J*=13.7Hz, 1H, PhC**H**$_b$), 7.24-7.40 (m, 10H, Ph**H**).

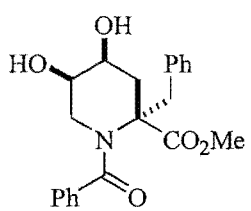
^{13}C NMR ($CDCl_3$) δ 15.7, 21.3, 28.9, 39.0, 43.3, 52.2, 63.8, 126.6, 127.9, 128.3, 129.4, 131.1, 136.7, 136.8, 171.4, 173.5.

FTIR (KBr) 2949, 1736, 1630cm^{-1}.

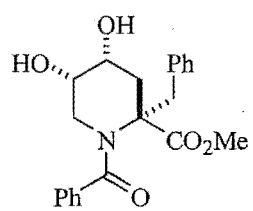
HRMS (EI) 337.1675 (M^+). $C_{21}H_{23}NO_3$ requires 337.1678.

$[\alpha]_D$ = +163.0°, c=1.0 $CHCl_3$.

Preparation of *cis*-(2*S*,4*S*,5*R*) and *cis*-(2*S*,4*R*,5*S*)-1-Benzoyl-2-benzyl-4,5-dihydroxy-piperidine-2-carboxylic acid methyl esters, 2.13a and 2.13b respectively.



2.13a



2.13b

Olefin (+)-**2.9** (45mg, 0.13mmol, 1 equiv) in acetone (0.03mL) was added, under nitrogen, to a combined solution of K_2OsO_4 (2.5mg, 0.05 equiv) in ^{t}BuOH (0.02mL), and NMO (17mg, 0.15mmol, 1.06 equiv) in water (0.08mL) and acetone (0.03mL). The reaction

mixture was stirred at rt for 24h. A slurry of magnesium silicate (17mg), and sodium dithionate (2.5mg) in water (0.1mL), was added to the reaction mixture. The magnesium silicate was filtered off and the filtrate was adjusted to pH 7 with 1N H_2SO_4. Acetone was removed under reduced pressure and the resulting aqueous phase acidified to pH 2. This was then saturated with NaCl and extracted with ethyl acetate (2x2mL). The organic extracts were combined, dried ($MgSO_4$) and the solvent removed under reduced pressure to give a crude mixture containing **2.13a** and **2.13b** in a ratio of 3:1 by ^{1}H NMR The residue was purified by radial chromatography (EA/CH_2Cl_2 1:1) to give a fraction containing the major *cis* isomer **2.13a**.

Data for **2.13a**:

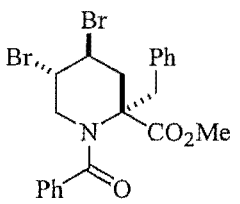
^{1}H NMR ($CDCl_3$ 300 MHz) δ 2.02 (dd *J*=14.2 and 4.9Hz, 1H, CC**H**$_a$CHOH), 2.27 (m, 2H, CC**H**$_b$CHOH and NC**H**$_a$CHOH), 3.04 (d *J*=13.7Hz, 1NC**H**$_b$CHOH

^{12}C NMR ($CDCl_3$) δ 35.0, 38.9, 49.0, 52.6, 63.6, 64.0, 65.0, 126.9, 127.1, 128.3, 128.6, 130.1, 131.0, 135.8, 136.0, 171.9, 173.0.

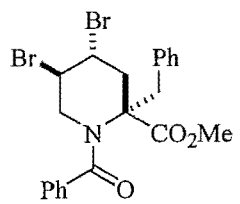
Selected data for **2.13b** (from crude):

^{1}H NMR ($CDCl_3$) δ 2.22 (m, 1H), 2.58 (d *J*=12.7Hz, 1H), 3.01 (d *J*=14.1Hz, 1H, C**H**$_a$Ph), 3.75 (dd *J*=13.7 and 3.4Hz, 1H), 3.86 (s, 3H, O**Me**), 3.99 (d *J*=14.1Hz, 1H, C**H**$_b$Ph).

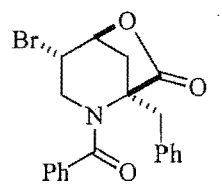
Preparation of (2*S*,4*S*,5*S*) and (2*S*,4*R*,5*R*)-1-Benzoyl-2-benzyl-4,5-dibromo-piperidine-2-carboxylic acid methyl esters and (2*S*,4*S*)-2-Benzoyl-1-benzyl-4-bromo-6-oxa-2-aza-bicyclo[3,2,1]octan-7-one, 2.14a, 2.14b and 2.16 respectively.



2.14a



2.14b



2.16

Bromine was added dropwise to a solution of olefin (+)-**2.9** (15mg, 0.045mmol) dissolved in CH_2Cl_2 (5mL) under nitrogen, until a permanent brown colour remained. The solution was stirred under N_2 for 3 h. Analysis of the crude ^{1}H NMR gave a mixture of **2.14a** and **2.14b** in the ratio of 4:1 by ^{1}H NMR. Purification by radial chromatography (EA/PE 1:3) yielded the major isomer **2.14a** (15mg, 52%).as a brown oil.

Data for **2.14a**:

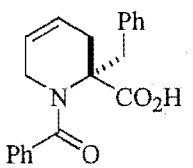
^{1}H NMR ($CDCl_3$) δ 2.40 (dd *J*=3.9 and 14.7Hz, 1H, CC**H**$_a$CHBr), 2.58 (**d *J*=13.2Hz**, 1H, CC**H**$_b$CHBr), 2.82 (dd *J*=5.1 and 15.9Hz, 1H, NC**H**$_a$CHBr), 3.04 (d *J*=13.7Hz, 1H, C**H**$_a$Ph), 3.60 (dd *J*=2.2 and 15.9Hz, 1H, NC**H**$_b$CHBr), 3.88 (s, 1H, OC**H**$_3$), 4.00 (m, 1H, NCH_2C**H**Br), 4.08 (d *J*=14.2Hz, 1H, C**H**$_b$Ph), 4.57 (m, 1H, CCH_2C**H**Br), 7.23-7.50 (m, 10H, Ph**H**).

Selected data for **2.14b** (from crude mixture):

^{1}H NMR ($CDCl_3$) δ 3.18 (d *J*=13.7Hz, 1H, C**H**$_a$Ph), 3.76 (s, 3H, O**Me**), 4.24 (d *J*=13.7Hz, 1H, C**H**$_b$Ph), 4.82 (m, 1H).

Attempted crystallisation by the diffusion of petroleum ether into a solution of **2.14a** dissolved in ethyl acetate, over a period of 6 months, gave **2.16**, the structure of which was solely determined on the basis of X-ray crystal structure analysis.

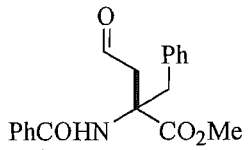
Preparation of (2*R*)-1-Benzoyl-2-benzyl-1,2,3,6-tetrahydro-piperidine-2-carboxylic acid 2.15.



Olefin (+)-**2.9** (50mg, 0.15 mmol, 1 equiv) was dissolved in MeOH (3mL) and hydrolysed with 1M aq. NaOH (0.3mL, 0.3mmol, 2 equiv) according to General Procedure G, to give acid **2.15** (44mg, 92%) as a white solid.

^{1}H NMR ($CDCl_3$ 500 MHz) δ 2.48 (m, 1H, CC**H**$_a$CH=), 2.80 (bd *J*=16.6Hz, 1H, C**H**$_b$CH=), 3.18 (d *J*=13.7Hz, 1H, PhC**H**$_a$), 3.61 (m, 1H, NC**H**$_a$CH=), 3.82 (d *J*=13.7Hz, 1H PhC**H**$_b$), 3.98 (dd *J*=5.4 and 16.6Hz, 1H, NC**H**$_b$CH=), 5.70 (m, 1H, CCH_2C**H**=), 5.97 (m, 1H, NCH_2C**H**=), 7.24-7.51 (m, 10H, Ph**H**).

Preparation of (2*S*)-2-Benzoylamino-2-benzyl-4-oxo-butyric acid methyl ester (-)-2.21.



Ozone was bubbled through a solution of (-)-**2.7** (1.8g, 5.57mmol, 1 equiv), stirred with solid $NaHCO_3$ (500mg) at -78°C in a mixture of CH_2Cl_2 (30mL) and MeOH (9mL) until the solution was distinctly blue in colour. The mixture was stirred for an additional 15min until the blue colour no longer remained, whereupon dimethyl sulfide (4 drops) was slowly added. The resulting colourless solution was strirred at -78°C for 10min, and then allowed to warm to rt and stirred for 6h. The mixture was filtered and the solvent removed to give a white residue. Purification by radial chromatography (EA/PE 4:1) gave aldehyde (-)-**2.21** (1.741g, 96%) as a colourless oil

^{1}H NMR ($CDCl_3$) δ 3.06 (d *J*=13.7Hz, 1H, C**H**$_a$CHO), 3.23 (d *J*=18.1Hz, 1H, PhC**H**$_a$), 3.79 (s, 3H, O**Me**), 3.91 (d *J*=13.7Hz, 1H, C**H**$_b$CHO), 4.29 (d *J*=18.1Hz, 1H, PhC**H**$_b$), 7.00 (m, 2H, Ph**H**), 7.16 (d *J*=6.8Hz, 1H, N**H**), 7.21 (m, 3H, Ph**H**), 7.40 (t *J*=7.3Hz, 2H, Ph**H**), 7.49 (t *J*=7.3Hz, 1H, Ph**H**), 7.65 (d *J*=7.3Hz, 2H, Ph**H**), 9.67 (s, 1H, C**H**O).

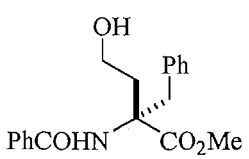
^{13}C NMR ($CDCl_3$) δ 40.6, 48.4, 52.7, 61.4, 126.7, 127.1, 128.1, 128.4, 129.5, 131.5, 134.4, 134.5, 166.9, 172.1, 198.7.

FTIR (KBr) 3406, 3032, 2955, 1744, 1659, 1601, 1582, 1516, 1489cm^{-1}

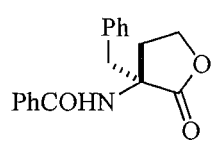
HRMS (ES) 348.1220 (M^++Na). $C_{19}H_{19}NO_4Na$ requires 348.1212.

$[\alpha]_D$ = -83.6°, c=1.0 $CHCl_3$.

Preparation of (2*S*)-2-Benzoylamino-2-benzyl-4-hydroxy butyric acid methyl ester 2.22 and (*3S*)-*N*-[3-Benzyl-2-oxo-tetrahydro-furan-3-yl]-benzamide 2.23.



2.22



2.23

$LiBH_4$ (24mg, 1.07mmol, 1equiv) was added to a solution of aldehyde (-)-**2.21** (354mg, 1.07mmol, 1equiv), in THF (5mL), at -78° C. The mixture was stirred at -78° C for 1h then

at rt for 1h. The solvent was removed under reduced pressure and the residue purified by radial chromatography (EA/PE 1:1) to give an initial fraction containing lactone **2.23**. Removal of the solvent gave **2.23** (189mg, 53%) as a white solid. Further elution gave a fraction containing alcohol **2.22**. Removal of the solvent gave **2.22** (144mg, 41%) as a colourless oil.

Data for **2.22**:

^{1}H NMR ($CDCl_3$) δ 2.76 (dt *J*=13.2 and 9.8Hz, 1H, CC**H**$_a$CH$_2$O), 2.87 (ddd *J*=13.2, 6.5 and 2.2Hz, 1H, CC**H**$_b$CH$_2$O), 3.25 (d *J*=13.2Hz, 1H PhC**H**$_a$), 3.33 (d *J*=13.2Hz, 1H, PhC**H**$_b$), 3.61 (dt *J*=7.3 and 9.8Hz, 1H, C**H**$_a$O), 4.35 (dt *J*=2.4 and 9.8Hz, 1H, C**H**$_b$O), 6.62 (s, 1H, N**H**), 7.27 (m, 2H, Ph**H**), 7.33 (m, 3H, Ph**H**), 7.44 (t *J*=7.5Hz, 2H, Ph**H**), 7.53 (t *J*=7.3Hz, 1H, Ph**H**), 7.73 (d *J*=7.3Hz, 2H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 32.9, 41.6, 60.1, 65.8, 127.0, 127.8, 128.5, 128.8, 130.0, 132.0, 133.2, 133.7, 166.7, 177.0.

HRMS (EI) 295.1206 (M^+). $C_{18}H_{17}NO_3$ requires 295.1208.

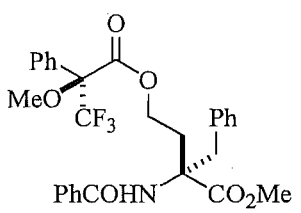
Data for **2.23**:

^{1}H NMR ($CDCl_3$) δ 2.37 (m, 1H, C**H**$_a$CH$_2$OH), 2.96 (m, 1H, C**H**$_b$CH$_2$OH), 3.22 (d *J*=13.4Hz, 1H, PhC**H**$_a$), 3.67 (m, 1H, C**H**$_a$OH), 3.75 (m, 1H, C**H**$_b$OH), 3.80 (s, 3H, O**Me**), 3.86 (d *J*=13.4Hz, 1H, PhC**H**$_b$), 7.02 (m, 2H, Ph**H**), 7.18 (m, 3H, Ph**H**), 7.24 (s, 1H, N**H**), 7.41 (t *J*=7.5Hz, 2H, Ph**H**), 7.50 (t *J*=7.3Hz, 1H, Ph**H**), 7.70 (dd *J*=8.3 and 1.0Hz, 2H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 37.8, 40.7, 52.7, 58.7, 64.1, 126.8, 126.9, 128.2, 128.6, 129.8, 131.6, 134.9, 135.9, 167.0, 174.2.

HRMS (EI) 327.1462 (M^+). $C_{19}H_{21}NO_4$ requires 327.1470.

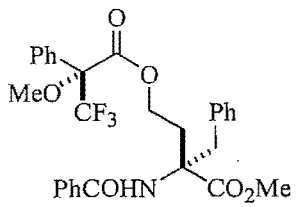
Preparation of (2*S*,2*S*)-2-Benzoylamino-2-benzyl-4-(3,3,3-trifluoro-2-methoxy-2-phenyl-propionyloxy)-butyric acid methyl ester 2.24.



Et_3N (10μL, .077mmol, 5equiv) and a solution of (*S*)-MTPA-Cl (3.5μL, 0.02mmol, 1.3equiv) in CH_2Cl_2 (0.15mL) was added, under argon, to a solution of alcohol **2.22** (5mg, 0.015mmol, 1equiv) and DMAP (2.05mg, 0.017mmol, 1.1equiv), in dry CH_2Cl_2 (0.5mL). The solution was stirred at rt for 10min, with the reaction followed by TLC to determine the reaction had gone to completion. The solvent was removed under reduced pressure to give **2.24** (8.3mg, quant.) which was analysed without further purification

^{1}H NMR ($CDCl_3$) δ 2.59 (m, 1H, CC**H**$_a$CH$_2$O), 3.12 (d *J*=13.7Hz, 1H, PhC**H**$_a$), 3.21 (dt *J*=4.9 and 19.5Hz, 1H, CC**H**$_b$CH$_2$O), 3.50 (s, 3H, CO**Me**), 3.67 (s, 3H, CO$_2$**Me**), 3.89 (d *J*=13.7Hz, 1H, C**H**$_a$CO), 4.20 (m, 1H, C**H**$_a$O), 4.43 (m, 1H, C**H**$_b$O), 6.89 (s, 1H, N**H**), 6.95 (m, 2H, Ph**H**), 7.16 (m, 3H, Ph**H**), 7.31-7.63 (m, 10H, Ph**H**).

Preparation of (2*S*,2*R*)-2-Benzoylamino-2-benzyl-4-(3,3,3-trifluoro-2-methoxy-2-phenyl-propionyloxy)-butyric acid methyl ester 2.25.

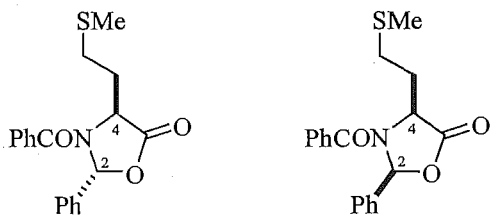


Et_3N (10μL, 0.077mmol, 5equiv) and a solution of (*R*)-MTPA-Cl (3.5μL, 0.02mmol, 1.3equiv) in CH_2Cl_2 (0.15mL) was added, under argon, to a solution of alcohol **2.22** (5mg, 0.015mmol, 1equiv) and DMAP (2.05mg, 0.017mmol, 1.1equiv), in dry CH_2Cl_2 (0.5mL). The solution was stirred at rt for 10min, with the reaction followed by TLC to determine the reaction had gone to completion. The solvent was removed under reduced pressure to give **2.25** (8.3mg, quant.) which was analysed without further purification

^{1}H NMR ($CDCl_3$) δ 2.50 (m, 1H, CC**H**$_a$CH$_2$O), 3.10 (d *J*=13.7Hz, 1H, PhC**H**$_a$), 3.24 (dt *J*=4.9 and 15.1Hz, 1H, CC**H**$_b$CH$_2$O), 3.46 (s, 3H, CO**Me**), 3.60 (s, 3H, CO$_2$**Me**), 3.89 (d *J*=13.7Hz, 1H, C**H**$_a$CO), 4.23 (m, 1H, C**H**$_a$O), 4.40 (m, 1H, C**H**$_b$O), 6.96 (m, 2H, Ph**H**), 7.03 (s, 1H, N**H**), 7.18 (m, 3H, Ph**H**), 7.35-7.67 (m, 10H, Ph**H**).

8.4 Experimental Described for Chapter Three

Preparation of (2*R*,4*S*) and (2*S*,4*S*)-3-Benzoyl-4-(2-methylsulfanyl-ethyl)-2-phenyl-oxazolidino-5-one, (+)-3.11 and 3.23 respectively.



(+)-**3.11** **3.23**

(*S*)-Methionine (5g, 33.55mmol) was reacted with 1M aq. NaOH (33.6mL, 33.55mmol, 1 equiv), and condensed with benzaldehyde (5.116mL, 50.33mmol, 1.5 equiv) according to Modified General Procedure A. Cyclisation with benzoyl chloride (3.895mL, 33.55mmol, 1 equiv) gave a crude mixture containing a 4:1 ratio, by ^{1}H NMR, of (+)-**3.11** and **3.23**. Recrystallisation from MeOH gave the *trans*-oxazolidinone (+)-**3.11** (7.21g ,63%) as a white solid.

Data for (+)-**3.11**:

mp 157-159° C (lit.[3] 157° C)

^{1}H NMR ($CDCl_3$) δ 2.06 (br s, 3H, SC**H**$_3$), 2.20-2.83 (m, 4H, C**H**$_2$C**H**$_2$SCH$_3$), 5.00 (br s, 1H, H4), 6.71 (br s, 1H, H2), 6.80-7.61 (m, 10H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 14.9, 28.7, 55.2, 91.1, 126.7, 126.9, 128.7, 129.9, 131.2, 134.9, 136.7, 170.6, 172.1.

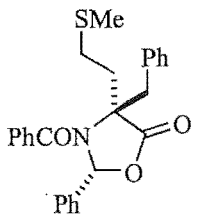
HMRS (ES) 342.1160 (M^++H). $C_{19}H_{20}NO_3S$ requires 342.1164.

$[\alpha]_D$ = +222° c=1.0 $CHCl_3$ (lit.[3] +280.4°).

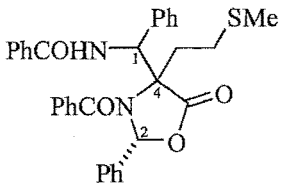
Selected data for **3.23** (from crude):

^{1}H NMR ($CDCl_3$) δ 4.91 (m, 1H, **H4**), 6.92 (s, 1H, **H2**).

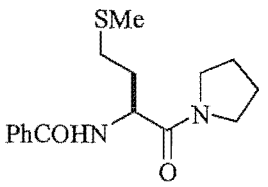
Preparation of (4*R*,2*R*)-3-Benzoyl-4-benzyl-4-(2-methylsulfanyl-ethyl)-2-phenyl-oxazolidin-5-one, (+)-3.12, *N*-{[3-Benzoyl-4-(2-methylsulfanyl-ethyl)-5-oxo-2-phenyl-oxazolidin-4-yl]-phenyl-methyl}-benzamide 3.30, and (*3S*)-*N*-[3-Methylsulfanyl-1-(pyrrolidine-1-carbonyl)-propyl]-benzamide 3.26.



3.12



3.30



3.26

Method A: Oxazolidinone (+)-**3.11** (5g, 14.7mmol, 1 equiv) was dissolved in dry THF (100mL) and cooled to -78°C under argon. LDA (9.53mL of a 2M solution in hexane, 19.1mmol, 1.3 equiv) was added dropwise and the resulting red solution was stirred at -78°C for 15min. Benzyl bromide (2.615mL, 22mmol, 1.5 equiv) was then added and the reaction mixture stirred at -78°C for 2 h then at rt overnight. The solution was quenched with sat. aq. NH_4Cl (20mL) and extracted with ether (3x20mL). The combined ether extracts were washed with water (20mL), dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by radial chromatography gave (+/-)-**3.12** (3.281g, 52%) as a white solid. Further elution gave the self-addition product **3.30** (76mg, 2%) as a white solid. An analytical sample was obtained by the diffusion of petroleum ether into a solution of (+/-)-**3.12** dissolved in ethyl acetate. Crystals suitable for X-ray crystallography were obtained (see Appendix for data).

Method B: Pyrrolidine (0.614mL, 7.33mmol, 1equiv) was dissolved in dry THF (5mL) and cooled to -50° C under argon. *n*-Butyl lithium (4.045mL of a 2M solution in THF, 8.07mmol, 1.1equiv) was added and the solution stirred at -20°C for 30min. The mixture was recooled to -50° C and a solution of oxazolidinone (+)-**3.11** (2.5g, 7.33mmol, 1 equiv), dissolved in dry THF (15 mL), added slowly. The solution was stirred at -50° C for 20min whereupon benzyl bromide (1.311mL, 11.02mmol, 1.5equiv) was added slowly. The reaction mixture was stirred at -50° C for 1h then allowed to warm to rt overnight. The dark yellow solution was quenched with saturated aqueous NH_4Cl solution (10mL) and the aqueous layer extracted with ether (3x20mL). The combined ether extracts were washed

with water (20mL) dried ($MgSO_4$), and the solvent removed under reduced pressure. Purification by radial chromatography (EA/PE 1:9) gave (+)-**3.12** (1.925g, 61%) as a white solid. Further elution (EA/PE 1:1) gave **3.26** (511mg, 29%) as a white solid.

Data for (+)-**3.12**:

^{1}H NMR ($CDCl_3$) δ 2.22 (s, 3H, S**Me**), 2.69-2.75 (m, 1H, C**H**$_a$CH$_2$SMe), 2.84-2.98 (m, 3H, C**H**$_b$C**H**$_2$SMe), 3.37 and 3.88 (dd *J*=13.4 Hz, 2H, C**H**$_2$Ph), 5.36 (s, 1H, C2**H**), 6.66 (d *J*=5.9 Hz, 2H, Ph**H**), 6.74 (d *J*=5.9 Hz, 2H, Ph**H**), 7.03 (m, 4H, Ph**H**), 7.13 (m, 2H, Ph**H**), 7.39 (m, 2H, Ph**H**), 7.44 (m, 3H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 15.4, 29.2, 36.9, 40.1, 68.7, 90.4, 125.6, 127.2, 127.9, 128.1, 128.2, 129.0, 130.1, 135.0, 136.1, 169.3, 173.5.

FTIR (KBr) 1787.9, 1654.8 cm^{-1}

HMRS (ES) 432.1637 (M^++H). $C_{26}H_{26}NO_3S$ requires 432.1633.

Micro. Calcd for $C_{26}H_{25}NO_3S$: C, 72.36; H, 5.84; N, 3.25; S, 7.43. Found: C, 72.39; H, 6.04; N, 3.47; S, 7.24.

$[\alpha]_D$ = +14.3°, c=1.0 $CHCl_3$.

Data for **3.30**:

^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3H, S**Me**), 2.75 (m, 2H, C**H**$_2$), 3.05 (m, 1H, C**H**$_a$), 3.30 (m, 1H, C**H**$_b$), 5.32 (s, 1H, **H2**), 6.15 (d *J*=9.2Hz, 1H, NHC**H**), 6.65-8.03 (m, 20H, Ph**H**), 9.56 (d *J*=8.8Hz, 1H, N**H**).

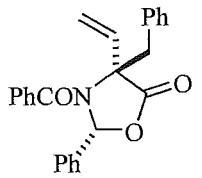
Data for **3.26**:

^{1}H NMR ($CDCl_3$) δ 1.85 (m, 2H, 2xNCH$_2$C**H**$_a$), 1.98 (m, 3H, 2xNCH$_2$C**H**$_b$ and C**H**$_a$CH$_2$SMe), 2.08 (m, 4H, S**Me** and C**H**$_b$CH$_2$SMe), 2.56 (m, 2H, C**H**$_2$SMe), 3.41 (m, 1H, NC**H**$_a$), 3.48 (m, 1H, NC**H**$_b$), 3.53 (ddd *J*=6.1, 10.1 and 16.9Hz, 1H, NC**H**$_a$'), 3.73 (ddd *J*=6.6, 10.0 and 16.8Hz, 1H, NHC**H**$_b$'), 5.07 (m, 3H, N**H** and Ph**H**), 7.45 (m, 1H, Ph**H**), 7.80 (t *J*=7.3Hz, 2H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 15.6, 24.0, 25.9, 30.2, 32.3, 46.0, 46.5, 50.3, 127.1, 128.3, 131.5, 133.7, 166.8, 169.8.

HRMS (ES) 307.1484 (M^++H). $C_{16}H_{23}N_2O_2S$ requires 307.1480.

Preparation of (4*R*,2*R*)-3-Benzoyl-4-benzyl-2-phenyl-4-vinyl-oxazolidin-5-one (+)-3.13.



Oxazolidinone (+)-**3.12** (1.312g, 3.04mmol, 1 equiv) was dissolved in acetic acid (6mL) and treated with H_2O_2 (0.289mL of a 50% w/w solution, 4.25mmol, 1.4 equiv) according to General Procedure Ia to give the intermediate sulfoxide **3.27** (1.26g, 94%), as a tan solid. The sulfoxide was then dissolved in degassed m-xylene and underwent oxidative elimination according to General Procedure Ib. Purification by radial chromatography (EA/PE 1:3) gave the vinyl oxazolidinone (+)-**3.13** (998mg, 93%) as a white solid. An analytical sample was obtained by the diffusion of petroleum ether into a solution of (+)-**3.13** dissolved in ethyl acetate. Crystals suitable for X-ray crystallography were obtained (see Appendix for data).

Data for **3.27**:

^{1}H NMR ($CDCl_3$) δ 2.70 (s, 3H, SO**Me**), 2.84-3.32 (m, 4H, C**H$_2$**C**H$_2$**SMe), 3.41 (d *J*=13.6Hz, 1H, C**H$_a$**Ph), 3.91 (br d *J*=13.6Hz,1H C**H$_b$**Ph), 5.50 (m, 1H, H2), 6.63 (m, 2H, Ph**H**), 6.73 (m, 2H, Ph**H**), 7.05 (m, 4H, Ph**H**), 7.16 (m, 2H, Ph**H**), 7.36 (m, 2H, Ph**H**), 7.44 (m, 3H, Ph**H**).

Data for (+)-**3.13**:

mp 148-149° C

^{1}H NMR ($CDCl_3$) δ 3.51 (d *J*=13.7Hz, 1H, C**H$_a$**Ph), 4.06 (d *J*=13.7Hz, 1H, C**H$_b$**Ph), 5.58 (d *J*=13.7Hz, =C**H$_a$**), 5.64 (m, 2H, =C**H$_b$** and C**H**=CH$_2$), 6.61 (d *J*=7.3Hz, 2H, Ph**H**), 6.73 (m, 3H, Ph**H**), 7.06 (m, 4H, Ph**H**), 7.16 (t *J*=7.3Hz, 1H, Ph**H**), 7.21 (t *J*=7.3Hz, 1H, Ph**H**), 7.41 (m, 4H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 40.6, 69.1, 90.4, 117.9, 125.8, 127.0, 127.8, 128.3, 128.9, 129.6, 129.8, 130.1, 135.1, 135.8, 135.9, 168.8, 171.5.

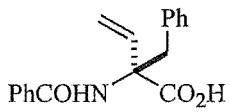
FTIR (KBr) 3034, 2939, 1801, 1649cm^{-1}.

HRMS (EI) 383.1521 (M^+). $C_{25}H_{21}NO_3$ requires 383.1521.

$[\alpha]_D$ = +139.0°, c=1.0 $CHCl_3$.

Micro. Calcd for $C_{25}H_{21}NO_3$. C, 78.2; H, 5.5; N, 3.7. Found: C, 77.8; H, 5.6; N, 3.7.

Preparation of (2*R*)-2-Benzoylamino-2-benzyl-but-3-enoic acid 3.14.

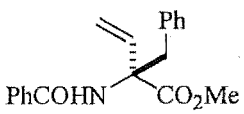


Oxazolidinone (+)-**3.13** (998mg, 2.61mmol, 1 equiv) was dissolved in MeOH (20mL) and hydrolysed with NaOH (209mg, 5.22mmol, 2 equiv) according to General Procedure B, to give acid **3.14** (760mg, 99%) as a white solid.

^{1}H NMR ($CDCl_3$) δ 3.54 (d *J*=13.7Hz, 1H, C**H**$_a$Ph), 3.74 (d *J*=13.7Hz, 1H, C**H**$_b$Ph), 5.38 (m, 2H, CH=C**H**$_2$), 6.18 (m, 1H, C**H**=CH$_2$), 6.87 (s, 1H, N**H**), 7.20-7.27 (m, 5H, Ph**H**), 7.42 (t *J*=7.3Hz, 2H, Ph**H**), 7.52 (t *J*=7.3Hz, 1H, Ph**H**), 7.67 (m, 2H, Ph**H**).

HRMS (ES) 296.1286 (M^++H). $C_{18}H_{18}NO_3$ requires 296.1287.

Preparation of (2*R*)-2-Benzoylamino-2-benzyl-but-3-enoic acid methyl ester (+)-3.15.



Acid **3.14** (760mg, 2.58mmol) was dissolved in ether (20mL), and esterified with ethereal diazomethane according to General Procedure C, to give methyl ester (+)-**3.15** (797mg, 100%) as a colourless oil.

^{1}H NMR 500MHz ($CDCl_3$) δ 3.43 (d *J*=13.7Hz, 1H, C**H**$_a$Ph), 3.83 (s, 1H, O**Me**), 3.92 (d *J*=13.7Hz, 1H, C**H**$_b$Ph), 5.33 (m, 2H, CH=C**H**$_2$), 6.20 (m, 1H, C**H**=CH$_2$), 7.00 (br s, 1H, N**H**), 7.09 (m, 2H, Ph**H**), 7.21 (m, 3H, Ph**H**), 7.42 (t *J*=7.8Hz, 2H, Ph**H**), 7.51 (t *J*=7.3Hz, 1H, Ph**H**), 7.71 (dd *J*=7.3 and 1.2Hz, 2H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 40.0, 53.0, 65.8, 116.3, 126.9, 127.1, 128.3, 128.6, 129.9, 130.0, 131.6, 134.7, 135.6, 136.2, 166.4, 172.2.

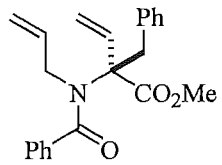
FTIR (KBr) 3464, 1749, 1645, 1543cm^{-1}.

HRMS (EI) 309.1365 (M^+). $C_{19}H_{19}NO_3$ requires 309.1359.

$[\alpha]_D$= +54.1°, c=1.0 $CHCl_3$.

Micro. Calcd for $C_{19}H_{19}NO_3$. C, 73.7; H, 6.2; N, 4.5. Found: C, 73.3; H, 6.2; N, 4.4.

Preparation of (2*R*)-2-(Allyl-benzoyl-amino)-2-benzyl-but-3-enoic acid methyl ester (-)-3.16.



Methyl ester (+)-**3.15** (612mg, 1.98mmol, 1 equiv) was dissolved in DMF (20mL) and reacted with allyl bromide (0.514mL, 5.94mmol, 3 equiv) and NaH (238mg of 60% in oil, 5.94mmol, 3 equiv) according to General Procedure D. Purification by radial chromatography (EA/PE 15/85) gave of 311mg (51%) of starting material (+)-**3.15**. Further elution gave.diene (-)-**3.16** (214mg, 31%) as a clear yellow oil.

^{1}H NMR ($CDCl_3$ at 500MHz) δ 3.33 (d *J*=13.4Hz, 1H, C**H**$_a$Ph), 3.38 (m, 1H, NC**H**$_a$), 3.64 (m, 1H, NC**H**$_b$), 3.81 (s, 3H, O**Me**), 4.03 (d *J*=13.4Hz, 1H, C**H**$_b$Ph), 4.99 (m, 2H, NCH_2CH=C**H**$_2$), 5.34 (m, 1H, NCH_2C**H**=CH_2), 5.36 (d *J*=3.4Hz, 1H, CCH=C**H**$_a$), 5.39 (d *J*=3.4Hz, 1H, CCH=C**H**$_b$), 6.22 (m, 1H, CC**H**=CH_2), 7.24-7.42 (m, 10H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 39.6, 50.7, 52.3, 70.1, 116.6, 117.3, 126.4, 126.9, 128.1, 128.1, 129.7, 131.0, 134.9, 135.8, 136.2, 136.5, 171.8, 173.0.

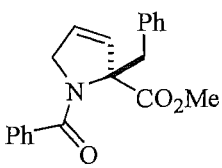
FTIR (KBr) 3395, 1736, 1624cm^{-1}.

HRMS (ES) 350.1763 (M^++H). $C_{22}H_{24}NO_3$ requires 350.1756.

Micro. Calcd for $C_{22}H_{23}NO_3$. C, 75.6; H, 6.6; N, 4.0. Found: C, 75.6; H, 6.5; N, 4.0,

$[\alpha]_D$ = -62.8°, c=1.0 $CHCl_3$.

Preparation of (2*R*)-1-Benzoyl-2-benzyl-2,5-dihydro-1H-pyrrole-2-carboxylic acid methyl ester (-)-3.17.



Catalyst **1.41** (18mg, 0.02mmol, 5mol%), dissolved in *dry degassed* benzene (0.5mL), was added to a solution of diene (-)-**3.16** (156mg, 0.45mmol), in *dry degassed* benzene (5mL) under argon, according to General Procedure E. The mixture was then stirred at reflux for

16h. Purification by radial chromatography (EA/PE 1:3 gave (-)-**3.17** (133mg, 94%) as a white solid. An analytical sample was obtained by the diffusion of petroleum ether into a solution of (-)-**3.17** dissolved in ethyl acetate. Crystals suitable for X-ray crystallography were obtained (see Appendix for data).

^{1}H NMR ($CDCl_3$) δ 3.29 (d *J*=13.7Hz, 1H, PhC**H**$_a$), 3.47 (d *J*=15.2Hz, 1H, NC**H**$_a$), 3.83 (s, 3H, O**Me**), 3.93 (d *J*=15.2Hz, 1H, NCH$_b$), 3.99 (d *J*=13.7Hz, 1H, PhC**H**$_b$), 5.71 (s, 2H, CH_2C**H**=CH and CH_2CH=C**H**), 7.19-7.40 (m, 10H, Ph**H**),

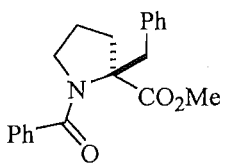
^{13}C NMR ($CDCl_3$) δ 38.0, 52.7, 56.9, 76.6, 126.4, 126.5, 127.6, 128.3, 129.3, 129.8, 130.7, 136.4, 136.5, 169.1, 171.6.

FTIR (KBr) 2951, 1738, 1645cm^{-1}.

HRMS (ES) 322.1444 (M^++H). $C_{20}H_{20}NO_3$ requires 322.1443.

$[\alpha]_D$ = -116.6°, c=1.0 $CHCl_3$

Preparation of (2*R*)-1-Benzoyl-2-benzyl-pyrrolidine-2-carboxylic acid methyl ester (-)-3.21.



Olefin (-)-**3.17** (18mg, 0.06mmol) was dissolved in dry MeOH (1.5mL) and reacted with 10% palladium-on-carbon (3.6mg, 20% w/w), under a hydrogen atmosphere according to General Procedure F, to give (-)-**3.21** (17mg, 95%)as a white solid.

^{1}H NMR ($CDCl_3$) δ 1.32 (m, 1H, CH_2C**H**$_a$$CH_2$), 1.81 (m, 1H, CH_2C**H**$_b$$CH_2$), 2.07 (m, 1H, CC**H**$_a$), 2.22 (m, 1H, CC**H**$_b$), 2.82 (m, 1H, NC**H**$_b$), 3.13 (d *J*=13.7Hz, 1H, PhC**H**$_a$), 3.82 (s, 3H, O**Me**), 4.01 (d *J*=13.7Hz, 1H, PhC**H**$_b$), 7.24-7.46 (m, 10H, Ph**H**),

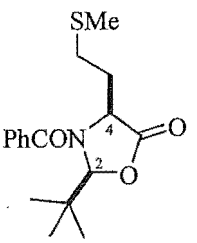
^{13}C NMR ($CDCl_3$) δ 23.6, 34.5, 37.4, 51.7, 52.5, 68.7, 126.7, 127.1, 128.1, 128.2, 130.1, 131.2, 136.7, 136.8, 169.3, 174.3.

FTIR (KBr) 2930, 1736, 1634, 1408cm^{-1}.

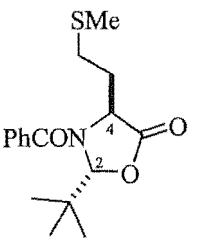
HRMS (EI) 323.1523 (M^+). $C_{20}H_{21}NO_3$ requires 323.1521.

$[\alpha]_D$= -106.5°, c=1.0 $CHCl_3$.

Preparation of (2*S*,4*S*) and (2*R*,4*S*)-3-Benzoyl-4-(2-methylsulfanyl-ethyl)-2-*tert*-butyl-oxazolidino-5-one, (+)-3.24 and 3.25 respectively.



(+)-**3.24**



3.25

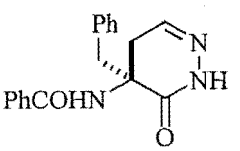
(*S*)-Methionine (6g, 40.2mol) was treated with 1M aq. NaOH (40.2mL, 40.2mmol, 1 equiv), and condensed with pivaldehyde (6.56mL, 60.3mmol, 1.5 equiv) according to Modified General Procedure A. Cyclisation with benzoyl chloride (4.68mL, 40.2mmol, 1 equiv) gave a crude mixture containing a 5:1 ratio, by ^{1}H NMR, of. (+)-**3.24** and **3.25**. Recrystallisation of the residue from MeOH gave the *cis*-5-oxazolidinone (+)-**3.24** (9.175g, 71%) as white needles. Crystals suitable for X-ray crystallography were obtained (see Appendix for data).

mp 123-125° C, (lit.[6] 126.2-126.6°).

^{1}H NMR ($CDCl_3$) δ 1.03 (s, 9H, C(C**H**$_3$)$_3$), 1.87 (s, 3H, SC**H**$_3$), 2.03 (m, 1H, C**H**$_a$CH$_2$S), 2.19 (m, 1H, C**H**$_b$CH$_2$S), 2.37 (m, 1H, C**H**$_a$S), 2.56 (m, 1H, C**H**$_b$S), 4.18 (dd *J*=3.4 and 10.7Hz, 1H, **H4**), 6.09 (s, 1H, **H2**), 7.40 (m, 2H, Ph**H**), 7.45-7.53 (m, 3H, Ph**H**).

8.5 Experimental Described for Chapter Four

Preparation of (*4S*)-*N*-(4-Benzyl-3-oxo-2,3,4,5-tetrahydro-pyridazin-4-yl)-benzamide (-)-4.12.



Hydrazine monohydrate (0.362mL, 7.24mmol ,1.3equiv) was added to a solution of aldehyde (-)-**2.19** (1.7g, 5,23mmol, 1 equiv) in THF (30mL), and the resulting solution was stirred at rt for 10min and then refluxed for 2 days. The mixture was cooled to rt and the

solvent removed to give a white solid. The solid was dissolved in ethyl acetate (10mL), filtered through a silica plug, and the solvent removed to yield (-)-**4.12** (1.378g, 85%) as a white solid.

mp 189-192° C

^{1}H NMR ($CDCl_3$) δ 2.90 (d *J*=18.2Hz, 1H, C**H**$_a$CH), 3.04 (d *J*=13.7Hz, 1H, PhC**H**$_a$), 3.58 (d *J*=13.7Hz, 1H, PhC**H**$_b$), 3.90 (dd *J*=4.6 and 18.2Hz, 1H, C**H**$_b$CH), 7.07 (m, 2H, Ph**H**), 7.19 (s, 1H, C**H**=N), 7.21-7.26 (m, 4H, PhCON**H** and Ph**H**), 7.42 (t *J*=7.6Hz, 2H, Ph**H**), 7.51 (t *J*=7.3Hz, 1H, Ph**H**), 7.70 (d *J*=7.8Hz, 2H, Ph**H**), 8.79 (brs, 1H, N=N**H**).

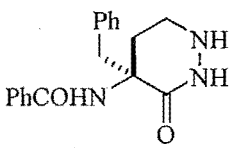
^{13}C NMR ($CDCl_3$) δ 34.1, 39.0, 56.3, 126.9, 127.3, 128.2, 128.5, 130.0, 131.8, 134.1, 134.4, 145.6, 167.1, 167.2.

FTIR (KBr) 3387, 3229, 3125, 2878, 1686, 1647, 1601, 1582, 1516, 1489cm^{-1}

HRMS (ES) 308.1395 (M^++H). $C_{18}H_{22}N_3O_2$ requires 308.1399.

$[\alpha]_D$ = -128.4°, c=1.0 $CHCl_3$.

Preparation of (*4S*)-*N*-(4-Benzyl-3-oxo-hexahydro-pyridazin-4-yl)-benzamide (-)-4.13.



A small crystal of methyl orange was added to a solution of hydrazone (-)-**4.12** (1.55g, 5.05mmol) dissolved in MeOH (40mL) at 0°C, causing the solution to turn yellow. Drops of 2N aqueous HCl in MeOH were added until the solution turned red. $NaBH_3CN$ (330mg, 5.3mmol, 1.05 equiv), was added slowly. Whenever the colour of the reaction mixture started to turn yellow during addition, drops of 2N aqueous HCl in MeOH were added immediately to restore to red colour. The reaction was stirred at 0°C for 3h then allowed to warm to rt overnight. The solvent was removed under reduced pressure, the red residue taken up in ethyl acetate and washed with saturated aqueous $NaHCO_3$ (2x20mL), water (2x20mL) and dried over $MgSO_4$. The solvent was removed to give the product as a white foam. Purification by radial chromatography (EA/PE 1:3) yielded (-)-**4.13** (1.135g, 73%).as a sticky white solid.

^{1}H NMR ($CDCl_3$) δ 2.51 (m, 2H, C**H**$_2$N), 2.83 (m, 1H, C**H**$_a$CH$_2$N), 3.15 (m, 1H, 1H, C**H**$_b$CH$_2$N), 3.19 (d *J*=13.2Hz, 1H, PhC**H**$_a$), 3.53 (d *J*=13.2Hz, 1H, PhC**H**$_b$), 6.99 (brs, 1H, N**H**), 7.23 (s, 1H, NH), 7.26 (m, 2H, PhH), 7.31-7.35 (m, 3H, PhH), 7.42 (t *J*=7.5Hz, 2H, PhH), 7.50 (m, 1H, PhH), 7.20 (d *J*=8.3Hz, 2H, PhH).

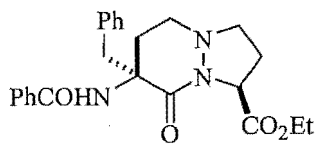
^{13}C NMR ($CDCl_3$) δ 34.4, 43.6, 44.5, 57.7, 127.0, 127.3, 128.3, 128.5, 130.3, 131.6, 133.8, 134.8, 166.8, 173.2.

FTIR (KBr) 3248, 3063, 2936, 1649, 1638, 1580, 1508, 1483cm^{-1}

HRMS (ES+) 310.1549 (M^++H). $C_{18}H_{20}N_3O_2$ requires 310.1556.

$[\alpha]_D$ = -25.7°, c=1.0 $CHCl_3$.

Preparation of (*7S,1S*)-*7*-Benzoylamino-7-benzyl-8-oxo-hexahydro-pyrrazolo[1,2-a]pyridazine-1-carboxylic acid ethyl ester 4.14.



Formaldehyde (29μL of 37% aqueous solution, 0.33mmol, 1.1 equiv) was added to a stirred solution of cyclic hydrazide (-)-**4.13** (100mg, 0.3mmol, 1 equiv), dissolved in ethyl acrylate (5mL) at 90°C, and the mixture then refluxed at 100-110° C for 4h. The mixture was then cooled to rt and the solvent removed under reduced pressure. Purification by radial chromatography (acetone/$CHCl_3$ 1:20) yielded **4.14** (26mg, 21%) as a colourless oil.

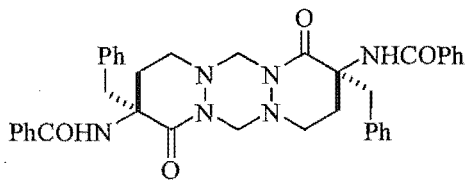
^{1}H NMR ($CDCl_3$) δ 1.28 (t *J*=7.3Hz, 3H, CO_2CH_2C**H**$_3$), 2.25 (m, 1H, C**H**$_a$CHCO$_2$Et), 2.55 (m, 2H, C**H**$_b$CHCO$_2$Et and CC**H**$_a$CH$_2$N), 3.00 (ddd *J*=6.3, 9.3 and 18.6Hz, 1H, C**H**$_a$CH$_2$CHCO$_2$Et), 3.04 (m, 3H, CC**H**$_b$CH$_2$N and CCH$_2$C**H**$_2$N), 3.21 (m, 1H, C**H**$_b$CH$_2$CHCO$_2$Et), 3.57 (d *J*=13.2Hz, 1H, PhC**H**$_a$), 3.68 (d *J*=13.2Hz, 1H, PhC**H**$_b$), 4.22 (q *J*=7.3Hz, 2H, CO_2C**H**$_2$CH$_3$), 4.60 (dd *J*=2.6 and 9.0Hz, 1H, C**H**CO$_2$CH$_2$CH$_3$), 7.05 (m, 2H, Ph**H** and N**H**), 7.20 (m, 3H, Ph**H**), 7.38 (m, 3H, Ph**H**), 7.47 (t *J*=7.3Hz, 1H, Ph**H**), 7.72 (d *J*=7.3Hz, 2H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 14.1, 28.9, 34.0, 41.2, 52.1, 54.3, 57.5, 59.5, 61.8, 126.9, 127.0, 128.1, 128.4, 130.0, 131.4, 134.8, 136.5, 165.5, 166.7, 170.2.

FTIR (KBr) 2939, 1736, 1649cm^{-1}

HRMS (ES+) 422.2075 (M^++H). $C_{24}H_{28}N_3O_4$ requires 422.2080.

Preparation of 4.16.



Formaldehyde (27μL of 37% aqueous solution, 0.31mmol, 1.1 equiv) was added to a stirred solution of cyclic hydrazide (-)-**4.13** (94mg, 0.28mmol, 1 equiv), dissolved in ethyl acrylate (5mL) at 80°C, and the mixture then refluxed at 80-90° C for 4h. The mixture was then cooled to rt and the solvent removed under reduced pressure. Purification by radial chromatography (acetone/$CHCl_3$ 1:20) yielded **4.16** (41mg, 45%) as a white solid.

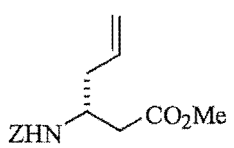
^{1}H NMR ($CDCl_3$) d 2.62 (m, 1H, CH_2CH_aN), 2.74 (m, 1H, CH_2CH_bN), 3.36 (m, 3H, CH_2CH_2N and CH_aPh), 3.60 (d *J*=13.7Hz, 1H, CH_bPh), 4.42 (m, 1H, NCH_aN), 5.09 (d *J*=10.2Hz, 1H, NCH_bN), 6.94 (s, 1H, NH), 7.20-7.67 (m, 10H, PhH).

^{13}C NMR ($CDCl_3$) d 28.8, 42.3, 46.1, 58.6, 64.5, 126.9, 127.4, 128.4, 128.5, 130.2, 131.6, 134.3, 135.1, 166.7, 170.0.

HRMS (ES+) 643.3035 (M^++H). $C_{38}H_{39}N_6O_4$ requires 643.3033.

8.6 Experimental Described in Chapter Five

Preparation of (3*R*)-3-Benzyloxycarbonylamino-hex-5-enoic acid methyl ester (+)-5.25.



Method A: (+/-)-(**3*R***)-**5.25:** Et_3N (2.794mL, 20.4mmol, 1 equiv) and $ClCO_2Et$ (1.920mL, 20.4mmol, 1 equiv) were reacted with (+/-)-*N*-Cbz-Allyl glycine (5g, 20.1mmol, 1 equiv) according to General Procedure Ja. The resulting diazoketone was dissolved in dry MeOH (80mL) and reacted with silver benzoate (506mg, 2.21mmol, 0.11 equiv) dissolved in Et_3N

(8.103mL, 58.23mmol, 2.9 equiv), according to General Procedure Jb. Purification by column chromatography (EA/PE 1:3) gave (+/-)-**5.25** (5.254g, 94%) as a colourless oil.

Method B: (+)-(*3R*)-*5*.**25:** Et_3N (0.10mL, 0.72mmol, 1.2 equiv), DPPA (0.129mL, 0.59mmol, 1 equiv) and benzyl alcohol (0.186mL, 1.80mmol, 3 equiv) were reacted with (+)-**5.41b** (103mg, 0.59mmol) dissolved in toluene (6mL), according to Modified Procedure M, to give (+)-**5.25** (117mg, 71%) as a colourless oil.

Method C: (+)-**5.25:** Ethereal diazomethane was added to a solution of **5.44** (80mg, 0.38mmol, 1 equiv) dissolved in ether (3mL), according to General Procedure C, to give (+)-**5.25** (83mg, 100%) as a colourless oil.

^{1}H NMR ($CDCl_3$) δ 2.34 (m, 2H, C**H**$_2$CH=CH_2), 2.56 (bd *J*=5.4Hz, 2H, C**H**$_2$$CO_2$Me), 3.67 (s, 3H, O**Me**), 4.06 (m, 1H, **αH**), 5.08 (m, 4H, PhC**H**$_2$ and =C**H**$_2$), 5.22 (m, 1H, N**H**), 5.74 (m, 1H, C**H**=CH_2), 7.35 (m, 5H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 37.92, 38.55, 47.49, 51.61, 66.53, 118.35, 127.93, 127.97, 128.38, 133.70, 136.39, 155.62, 171.77.

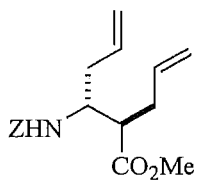
FTIR (KBr) 3339, 1724, 1643, 1529cm^{-1}.

HRMS (EI) 278.1391 (M^++H). $C_{15}H_{19}NO_4$ requires 278.1392.

Micro. Calcd for $C_{15}H_{19}NO_4$. C, 64.96; H, 6.91; N, 5.05. Found C, 64.67; H, 6.79; N, 5.33.

$[\alpha]_D$ = +4.2°, c=2.0 $CHCl_3$ (lit.[7] +4.7°)..

Preparation of (*2R,3R*)-*2*-Allyl-3-benzyloxycarbonylamino-hex-5-enoic acid methyl ester (+)-5.26.



Anhydrous LiCl (444mg, 10.83mmol, 3 equiv), LDA (3.971mL of a 2M solution in THF, 7.94mmol, 2.2 equiv), and allyl bromide (1.258mL, 14.44mmol, 4 equiv) were reacted with a solution of (+/-)-**5.25** (1g, 3.61mmol, 1 equiv), dissolved in THF (20mL) at -78° C under nitrogen, according to General Procedure K. Purification by radial chromatography (EA/PE 1:4) gave (+/-)-**5.26** (674mg, 59%) as a colourless oil.

Anhydrous LiCl (294mg, 7.17mmol, 3 equiv), LDA (2.629mL of a 2M solution in THF, 5.25mmol, 2.2 equiv), and allyl bromide (0.833mL, 9.56mmol, 4 equiv) were reacted with (+)-**5.25** (662mg, 2.39mmol, 1 equiv), dissolved in THF (12mL) at -78° C, according to General Procedure K. Purification by radial chromatography (EA/PE 1:4) gave (+)-**5.26** (370mg, 49%) as a colourless oil.

^{1}H NMR ($CDCl_3$) δ 2.18-2.44 (m, 4H, 2x**CH$_2$**CH=CH$_2$), 2.69 (m, 1H, **CH**CO$_2$Me), 3.67 (s, 3H, OC**H$_3$**), 3.93 (m, 1H, NHC**H**), 5.02-5.13 (m, 6H, PhC**H$_2$** and 2xCH=C**H$_2$**), 5.63 (d *J*=9.8Hz, 1H, N**H**), 5.73 (m, 2H, 2xC**H**=CH$_2$), 7.35 (s, 5H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 34.2, 39.0, 47.5, 51.2, 51.6, 66.6, 117.5, 118.1, 127.9, 128.0, 128.4, 133.8, 134.4, 136.6, 156.1, 174.7.

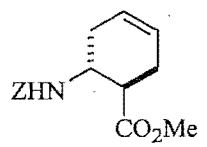
FTIR (KBr) 3344, 2953, 1717, 1643, 1506cm^{-1}.

HRMS (ES) 340.1536 (M^++Na). $C_{18}H_{23}NO_4Na$ requires 340.1525.

Micro. Calcd for $C_{18}H_{23}NO_4$. C, 68.12; H, 7.3; N, 4.41. Found: C, 68.37; H, 7.47; N, 4.53.

$[\alpha]_D$ = +8.3°, 1.0 CH_2Cl_2.

Preparation of (*6R,1R*)-*6*-Benzyloxycarbonylamino-cyclohex-3-enecarboxylic acid methyl ester (-)-5.27.



Catalyst **1.40** (46mg, 0.06mmol, 5mol%), dissolved in *dry degassed* benzene (0.5mL), was added to a solution of diene (+/-)-**5.26** (355mg, 1.12mmol), dissolved in *dry degassed* benzene (10mL) under argon, according to General Procedure E. The mixture was then stirred at reflux for 4h. Purification by radial chromatography (EA/PE 1:3) gave (+/-)-**5.27** (311mg, 96%) as a colourless oil.

Catalyst **1.42** (8mg, 0.01mmol, 5mol%), dissolved in *dry degassed* benzene (0.5mL), was added to a solution of diene (+)-**5.26** (59mg, 0.19mmol), dissolved in *dry degassed* benzene (10mL) under argon, according to General Procedure E. The mixture was then stirred at reflux for 4h. Purification by radial chromatography (EA/PE 1:3) gave (-)-**5.27** (49mg, 91%) as a colourless oil.

^{1}H NMR ($CDCl_3$) δ 1.99 (brd *J*=9.8Hz, 1H, NHCHC**H**$_a$CH=), 2.30 (dd *J*=12.2 and 5.9Hz, 1H, CH(C**H**$_a$CH=)CO_2Me), 2.49 (m, 2H, NHCHC**H**$_b$CH= and CH(C**H**$_b$CH=)CO_2Me), 2.72 (m, 1H, C**H**CO_2Me), 3.64 (s, 3H, O**Me**), 4.11 (m, 1H, NHC**H**), 4.90 (brs, 1H, N**H**), 5.08 (s, 2H, $PhCH_2$), 5.59 (m, 1H, $NHCHCH_2$C**H**=), 5.66 (m, 1H, $CH(CH_2$C**H**=)CO_2Me), 7.33 (m, 5H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 26.6, 31.0, 44.3, 47.8, 51.8, 66.6, 124.1, 124.9, 128.0, 128.1, 128.4, 136.4, 155.5, 173.9.

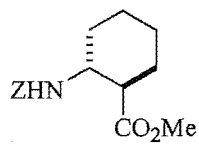
FTIR (KBr) 3339, 3032, 2930, 2853, 1732, 1520cm^{-1}.

HRMS (ES) 290.1402 (M^++H). $C_{16}H_{20}NO_4$ requires 290.1392.

Micro. Calcd for $C_{16}H_{19}NO_4$. C, 66.48; H, 6.62; N, 4.84. Found: C, 66.38; H, 6.83; N, 4.94.

$[\alpha]_D$ = –31.2°, c=1 $CHCl_3$ (lit.[8] 33.5°).

Preparation of (*2R,1R*)-*2*-Benzyloxycarbonylamino-cyclohexanecarboxylic acid methyl ester 5.28.



(+/-)-**5.28**: 10%-Palladium-on-carbon (5mg, 20% w/w), DIEA (0.072mL, 0.42mmol, 2.4 equiv), DMAP (6.2mg, 0.05mmol, 0.28 equiv) and benzylchloroformate (0.04mL, 0.27mmol, 1.6 equiv) were reacted, with (+/-)-**5.27** (50mg, 0.17mmol), dissolved in dry MeOH (3mL), according to General Procedure L, to give (+/-)-**5.28** (43mg, 86%) as a colourless oil.

(-)-**5.28**: 10%-Palladium-on-carbon (5mg, 20% w/w), DIEA (0.029mL, 0.17mmol, 2.4 equiv), DMAP (2.5mg, 0.02mmol, 0.28 equiv) and benzylchloroformate (0.016mL, 0.112mmol, 1.6 equiv) were reacted, with (-)-**5.27** (20mg, 0.07mmol), dissolved in dry MeOH (1.5mL), according to General Procedure L, to give (-)-**5.28** (15mg, 75%) as a colourless oil.

^{1}H NMR ($CDCl_3$) δ 1.13-1.44 (m, 3H), 1.56-1.81 (m, 3H), 1.90 (m, 1H), 2.06 (m, 1H), 2.27 (m, 1H), 3.61 (s, 3H, O**Me**), 3.73 (ddd *J*=20.5,11.3 and 4.2Hz, 1H), 4.08 (brs, 1H), 5.06 (s, 2H, PhC**H**$_2$), 7.27-7.37 (m, 5H, Ph**H**).

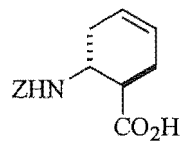
^{13}C NMR (CDCl3) δ 24.3, 24.6, 28.6, 32.8, 49.7, 51.7, 66.5, 128.0, 128.4, 136.6, 155.4, 174.3.

FTIR (KBr) 3331, 1733cm^{-1}.

HRMS (EI) 291.1474 (M^+). $C_{16}H_{21}NO_4$ requires 291.1471.

$[\alpha]_D$ = -18.4°, c=0.9 $CHCl_3$ (lit.[9] -18°).

Preparation of (*6R,1R*)-*6*-Benyloxycarbonylamino-cyclohex-5-enoic acid 5.29.

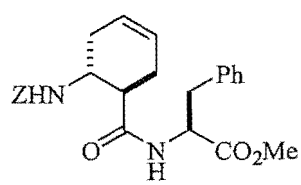


1M Aq. NaOH (0.34mL, 0.35mmol, 2 equiv) was added to a solution of (+/-)-**5.25** (50mg, 0.17mmol) dissolved in MeOH (3mL), and refluxed for 4h according to General Procedure G, to give (+/-)-**5.29** (47mg, 99%) as a yellow oil.

^{1}H NMR ($CDCl_3$) δ 2.00 (dd *J*=4.9 and 17.6Hz, 1H, NHCHC**H**$_a$CH=), 2.34 (bd *J*=16.1Hz, 1H, CH(C**H**$_a$CH=)CO_2H), 2.52 (m, 2H, NHCHC**H**$_b$CH= and CH(C**H**$_b$CH=)CO_2H), 2.78 (m, 1H, C**H**CO_2Me), 4.13 (m, 1H, NHC**H**), 5.07 (bs, 1H, PhC**H**$_a$), 5.11 (d *J*=12.3Hz, 1H, PhC**H**$_b$), 5.61 (m, 1H, NHCHCH_2C**H**=), 5.66 (m, 1H, CH(CH_2C**H**=)CO_2Me), 7.33 (m, 5H, Ph**H**).

HRMS (ES) 257.1052 (M^+-OH). $C_{15}H_{15}NO_3$ requires 257.1052.

Preparation of 2-[(6-Benzyloxycarbonylamino-cyclohex-3-enecarbonyl)-amino]-3-phenyl-propionic acid methyl esters 5.30a and 5.30b.



(*S*)-Phenylalanine methyl ester hydrochloride (35mg, 0.165mmol, 1.1 equiv), EDCI (39mg, 0.195mmol, 1.3 equiv), HOBt (32mg, 0.225mmol, 1.5 equiv) and DIEA (0.035mL, 0.165mmol, 1.1 equiv), were added to a solution of (+/-)-**5.28** (40mg, 0.15mmol, 1 equiv) dissolved in CH_2Cl_2 (5mL) and the solution stirred at rt for 2h. The mixture was washed with $NaHCO_3$ (10mL), NaCl (10mL), dried ($MgSO_4$), and the solvent removed under

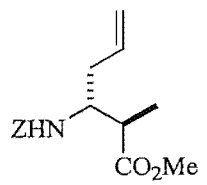
reduced pressure. Attempted purification by radial chromatography (EA/PE 1:1) gave a fraction containing **5.30a** and **5.30b** (58mg, 91%), in a ratio of 1:1 by ^{1}H NMR, that could not be separated further.

^{1}H NMR ($CDCl_3$) δ 2.20-2.37, (m, 2H, =CHC**H**$_2$CHCO), 2.68, *2.78* (m, 1H, CHC**H**CO) 2.99-3.13 (m, 2H, C**H**$_2$Ph), 3.64, *3.66* (s, 3H, O**Me**), 3.78, *3.87* (m, 1H, NHC**H**CH), *4.81*, 4.85 (m, 1H, C**H**CO$_2$Me), 5.01-5.22 (m, 3H, PhC**H**$_2$ and N**H**), 5.57-5.64 (m, 2H, C**H**=C**H**), 6.37, *6.48* (m, 1H, N**H**), 7.07-7.35 (m, 10H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 28.3, 31.1 (**C**H$_2$CH=CH**C**H$_2$), *37.6*, *37.9* (**C**H$_2$Ph), *45.6*, *46.0* (CH**C**HCO), *48.5*, *49.0* (NH**C**HCH), *52.2*, *52.2* (O**C**H$_3$), *53.0*, *53.3* (**C**HCO$_2$Me), *66.6*, *66.8* (PhCH$_2$O), *124.4*, *124.6* (CH=**C**H), *125.0*, *125.2* (**C**H=CH), *127.0*, *127.0*, *127.1*, *127.9*, *128.0*, *128.1*, *128.4*, *128.5*, *128.5*, *128.6*, *129.1*, *129.1* (Aromatic **C**H), *135.9*, *136.1*, *136.3*, *136.4* (Aromatic **C**), 156.0 (CH**C**ONH), 172.0 (**C**O), 173.2 (**C**O).

Micro. Calcd for $C_{16}H_{19}NO_4$. C, 68.79; H, 6.47; N, 6.42; Found: C, 68.84; H, 6.20, N, 6.39.

Preparation of (2*R*,3*R*)-2-Methyl-3-benzyloxycarbonylamino-hex-5-enoic acid methyl ester (+/-)-5.32a.



Anhydrous LiCl (266mg, 6.5mmol, 3 equiv), LDA (2.383mL of a 2M solution in THF, 4.76mmol, 2.2 equiv), and MeI (0.543mL, 8.66mmol, 4 equiv) were reacted with a -78° C solution of (+/-)-**5.25** (600mg, 2.17mmol) dissolved in THF (10mL) under nitrogen, according to General Procedure K. Purification by radial chromatography (EA/PE 1:4) gave (+/-)-**5.32a** (543mg, 86%) as a colourless oil.

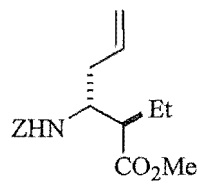
^{1}H NMR ($CDCl_3$) δ 1.22 (d *J*=6.Hz, 3H, CC**H**$_3$), 2.26 (m, 2H, C**H**$_2$CH=CH$_2$), 2.73 (m, 1H, C**H**CH$_3$), 3.67 (s, 3H, OC**H**$_3$), 3.86 (m, 1H, NC**H**), 5.08 (m, 4H, OC**H**$_2$ and CH=C**H**$_2$), 5.50 (d *J*=9.3Hz, 1H, N**H**), 5.75 (m, 1H, C**H**=CH$_2$), 7.35 (m, 5H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 14.7, 38.2, 51.5, 52.8, 66.4, 117.8, 127.8, 127.9, 128.3, 133.9, 136.5, 156.2, 175.4.

FTIR (KBr) 3317, 2952, 1717, 1642, 1506, 1206cm^{-1}.

Micro. Calcd for $C_{16}H_{21}NO_4$. C, 65.96; H, 7.26; N, 4.81. Found: C, 66.05; H, 7.33; N, 4.99.

Preparation of (3 *R*,2*R*)-3-Benzyloxycarbonylamino-2-ethyl-hex-5-enoic acid methyl ester (+/-)-5.32b.



Anhydrous LiCl (193mg, 4.7mmol, 3 equiv), LDA (1.727mL of a 2M solution in THF, 3.45mmol, 2.2 equiv), and EtI (0.503mL, 6.28mmol, 4 equiv) were reacted with a -78° C solution of (+/-)-**5.25** (435mg, 1.57mmol) dissolved in THF (10mL) under nitrogen, according to General Procedure K. Purification by radial chromatography (EA/PE 15/85) gave (+/-)-**5.32b** (302mg, 63%) as a colourless oil.

^{1}H NMR ($CDCl_3$) δ 0.93 (t *J*=7.2Hz, 3H, CH_2CH_3), 1.58 (m, 1H, CH_aCH_3), 1.71 (m, 1H, CH_bCH_3), 2.17-2.27 (m, 2H, $CH_2CH{=}CH_2$), 2.51 (m, 1H, $CHCO_2Me$), 3.68 (s, 3H, OMe), 3.93 (m, 1H, NHCH), 5.05-5.13 (m, 4H, $CH{=}CH_2$ and $PhCH_2O$), 5.62 (d *J*=9.8Hz, 1H, NH), 5.75 (m, 1H, $CH{=}CH_2$), 7.35 (m, 5H, PhH).

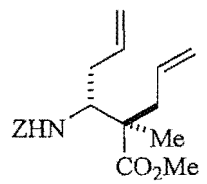
^{13}C NMR ($CDCl_3$) δ 11.7, 23.0, 38.7, 49.2, 51.0, 51.3, 66.3, 117.8, 127.7, 127.8, 128.2, 133.8, 136.5, 156.1, 175.2.

FTIR (KBr) 3342, 2930, 1720, 1643, 1502, 1227 cm^{-1}.

HRMS (ES) 306.1711 (M^++H). $C_{17}H_{24}NO_4$ requires 306.1705.

Micro. Calcd for $C_{17}H_{23}NO_4$. C, 66.86; H, 7.59; N, 4.59: Found: C, 66.14; H, 7.31; N, 4.59.

Preparation of (2*R*,3*R*)-2-Allyl-3-benzyloxycarbonylamino-2-methyl-hex-5-enoic acid methyl ester (+/-)-5.33a.



Anhydrous LiCl (25mg, 0.62mmol, 3 equiv), LDA (0.226mL of a 2M solution in THF, 0.45mmol, 2.2 equiv), and allyl bromide (0.072mL, 0.84mmol, 4 equiv) were reacted with a -78° C solution of (+/-)-**5.32a** (60mg, 0.21mmol) dissolved in THF (1mL) under nitrogen, according to General Procedure K. Purification by radial chromatography (EA/PE 1:9) gave (+/-)-**5.33a** (32mg, 47%) as a colourless oil.

^{1}H NMR ($CDCl_3$) δ 1.19 (s, 3H, CC**H**$_3$), 1.93 (m, 1H, C3C**H**$_a$CH=CH$_2$), 2.24 (dd *J*=7.3 and 13.7Hz, 1H, C2C**H**$_a$CH=CH$_2$), 2.43 (m, 1H, C3C**H**$_b$CH=CH$_2$), 2.51 (dd *J*=7.3 and 13.7Hz, 1H, C2C**H**$_b$CH=CH$_2$), 3.65 (s, 3H, OC**H**$_3$), 3.80 (td *J*=3.4 and 10.8Hz, 1H, NC**H**), 5.07 (m, 6H, OC**H**$_2$ and 2 x CH=C**H**$_2$), 5.44 (d *J*=10.7Hz, 1H, N**H**), 5.79 (m, 2H, 2 x C**H**=CH$_2$), 7.30 (m, 5H, Ph**H**).

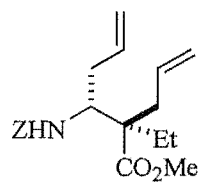
^{13}C NMR ($CDCl_3$) δ 19.4, 36.0, 41.3, 49.6, 51.7, 56.3, 66.5, 117.5, 118.8, 127.9, 127.9, 128.4, 133.0, 134.4, 136.6, 156.2, 175.9.

FTIR (KBr) 3348, 3076, 2980, 1724, 1641 cm^{-1}.

HRMS (ES) 332.1863 (M^++H). $C_{19}H_{25}NO_4$ requires 332.1862.

Micro. Calcd for $C_{19}H_{25}NO_4$. C, 68.86; H, 7.60; N, 4.23. Found: C, 68.80; H, 7.60; N, 4.23.

Preparation of (2*R*,3*R*)-2-Allyl-3-benzyloxycarbonylamino-2-ethyl-hex-5-enoic acid methyl ester (+/-)-5.33b.



Anhydrous LiCl (36mg, 0.89mmol, 3 equiv), LDA (0.324mL of a 2M solution in THF, 0.65mmol, 2.2 equiv), and allyl bromide (0.103mL, 1.2mmol, 4 equiv) were reacted with a -78° C solution of (+/-)-**5.32b** (90mg, 0.3mmol) dissolved in THF (1.5mL) under nitrogen, according to General Procedure K. Purification by radial chromatography (EA/PE 1:9) gave (+/-)-**5.33b** (41mg, 42%) as a colourless oil.

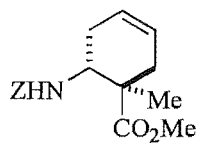
^{1}H NMR ($CDCl_3$) δ 0.89 (t *J*=7.5Hz, 3H, CH$_2$C**H**$_3$), 1.64 (m, 1H, C**H**$_a$CH$_3$), 1.78-1.96 (m, 2H, C**H**$_b$CH$_3$ and CHC**H**$_a$CH=CH$_2$), 2.35 (m, 2H, CHC**H**$_b$CH=CH$_2$ and CC**H**$_a$CH=CH$_2$), 2.52 (ddd *J*=6.8, 16.1 and 30.7Hz , 1H, CC**H**$_b$CH=CH$_2$), 3.68 (s, 3H, O**Me**), 3.97 (ddd

J=3.2, 11.3 and 21.2Hz, 1H, NHC**H**), 4.97-5.14 (m, 6H, 2xCH=C**H**$_2$ and PhC**H**$_2$O), 5.32 (d *J*=10.3Hz, 1H, N**H**), 5.55-5.77 (m, 2H, 2xC**H**=CH$_2$), 7.35 (m, 5H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 7.7, 24.3, 36.2, 36.3, 51.8, 52.9, 54.1, 66.6, 117.4, 118.3, 127.9, 128.0, 128.4, 133.7, 134.5, 136.7, 156.5, 175.8.

FTIR (KBr) 3350, 2930, 1720, 1643, 1502, 1223cm^{-1}.

Preparation of (6*R*,1*R*)-6-Benzyloxycarbonylamino-1-methyl-cyclohex-3-ene-carboxylic acid methyl ester (+/-)-5.34a

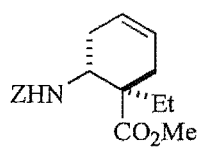


Catalyst **1.40** (3mg, 5mol%), dissolved in *dry degassed* benzene (0.5mL), was added to a solution of diene (+/-)-**5.33a** (25mg, 0.08mmol), dissolved in *dry degassed* benzene (1mL) under argon, according to General Procedure E. The mixture was then stirred at reflux for 4h. Purification by radial chromatography (EA/PE 1:3) gave (+/-)-**5.34a** 24mg, 96%) as a colourless oil.

^{1}H NMR ($CDCl_3$) δ 1.17 (s, 3H, C2C**H**$_3$), 1.89 (dd *J*=17.1 and 2Hz, 1H, C2C**H**$_a$), 1.95 (dd *J*=17.6 and 2Hz, 1H, NC**H**$_a$), 2.42 (d *J*=17.6Hz, 1H, NC**H**$_b$), 2.72 (d *J*=17.1, 1H, C2C**H**$_b$), 3.65 (s, 3H, OC**H**$_3$), 4.31 (m, 1H, C3**H**), 4.79 (d *J*=9.3Hz, 1H, N**H**), 5.09 (s, 2H, OC**H**$_2$), 5.56 (m, 1H, NCH$_2$CH=C**H**), 5.65 (m, 1H, NCH$_2$C**H**=CH), 7.35 (m, 5H, Ph**H**).

FTIR (KBr) 3346, 3032, 2932, 2851, 1728, 1526cm^{-1}.

Preparation of (6*R*,1*R*)-6-Benzyloxycarbonylamino-1-ethyl-cyclohex-5-enoic acid methyl ester (+/-)--5.34b.



Catalyst **1.40** (2.5mg, 5mol%), dissolved in *dry degassed* benzene (0.5mL), was added to a solution of diene (+/-)-**5.34b** (21mg, 0.06mmol) dissolved in *dry degassed* benzene (1mL) under argon, according to General Procedure E. The mixture was then stirred at reflux for

4h. Purification by radial chromatography (EA/PE 1:9) gave (+/-)-**5.34b** (18mg, 94%) as a colourless oil.

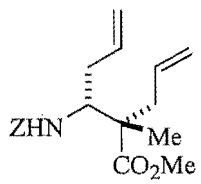
^{1}H NMR ($CDCl_3$) δ 0.81 (t *J*=7.8Hz, 3H, CH_2CH_3), 1.51 (ddd *J*=28.8, 14.2 and 7.3_Hz, 1H, CH_aCH_3), 1.77 (m, 2H, CH_bCH_3 and CH_aCCO_2Me), 2.02 (d *J*=18.6Hz, 1H, $CHCH_aCH$=), 2.38 (d *J*=18.6Hz, 1H, $CHCH_bCH$=), 2.75 (d *J*=18.6Hz, 1H, CH_bCCO_2Me), 3.68 (s, 3H, CO_2Me), 4.37 (d *J*=10.2Hz, 1H, NHCH), 4.86 (d *J*=10.2Hz, 1H, NH), 5.10 (dd *J*=21.5 and 2.2Hz, 2H, $PhCH_2$), 5.55 (m, 1H, $CHCH_2CH$=), 5.70 (m, 1H, $=CHCH_2CCO_2Me$), 7.34 (m, 5H, PhH).

^{13}C NMR ($CDCl_3$) δ 8.4, 28.6, 29.3, 30.5, 49.0, 49.8, 51.9, 66.9, 123.4, 125.9, 128.2, 128.5, 136.3, 156.3, 175.0.

FTIR (KBr) 3343, 3032, 2951, 1732, 1717, 1504, 1454, 1234cm^{-1}.

HRMS (ES) (M^++H). $C_{18}H_{24}NO_4$ requires 318.1705.

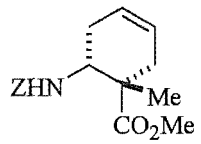
Preparation of (2*S*,3*R*)-2-Allyl-3-benzyloxycarbonylamino-2-methyl-hex-5-enoic acid methyl ester (+/-)-5.35.



Anhydrous LiCl (43mg, 1.0mmol, 3 equiv), LDA (0.381mL of a 2M solution in THF, 0.76mmol, 2.2 equiv), and MeI (0.086mL, 1.4mmol, 4 equiv) were reacted with a -78° C solution of (+/-)-**5.26** (110mg, 0.35mmol) dissolved in THF (1.8mL) under nitrogen, according to General Procedure K. Purification by radial chromatography (EA/PE 15:85) gave (+/-)-**5.35** (71mg, 68%) as a colourless oil.

^{1}H NMR ($CDCl_3$) δ 1.14 (s, 3H, CH_3), 1.99 (m, 1H, $C3CH_aCH=CH_2$), 2.19-2.33 (m, 2H, $C2CH_aCH=CH_2$ and $C3CH_bCH=CH_2$), 2.56 (dd *J*=6.6 and 23.9Hz, 1H, $C2CH_bCH=CH_2$), 3.67 (s, 3H, CO_2Me), 4.00 (td *J*=3.4 and 10.7Hz, 1H, NHCH), 4.93 (d *J*=10.3Hz, 1H, NH), 4.97-5.13 (m, 6H, $PhCH_2$ and 2 x $CH=CH_2$), 5.73 (m, 2H, 2 x $CH=CH_2$), 7.29-7.37 (m, 5H, PhH).

Preparation of (6*R*,1*S*)-6-Benzyloxycarbonylamino-1-methyl-cyclohex-3-ene-carboxylic acid methyl ester (+/-)-5.36.



Catalyst **1.40** (1.7mg, 5mol%), dissolved in *dry degassed* benzene (0.5mL), was added to a solution of diene (+/-)-**5.35** (14mg, 0.04mmol, 1 equiv), dissolved in *dry degassed* benzene (1mL) under argon, according to General Procedure E. The mixture was then stirred at reflux for 4h. Purification by radial chromatography (EA/PE 1:3) gave (+/-)-**5.36** (12mg, 91%) as a colourless oil.

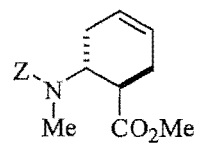
^{1}H NMR ($CDCl_3$) δ 1.27 (s, 3H, CCH_3), 2.06 (dd *J*=2.4 and 21.0Hz, 1H, =CHC**H**$_a$C), 2.12 (m, 1H, CHC**H**$_a$CH=), 2.37 (m, 1H, CHC**H**$_b$CH=), 2.69 (dd *J*=4.4 and 17.1Hz, 1H, =CHC**H**$_a$C), 3.66 (s, 3H, O**Me**), 3.94 (m, 1H, NHC**H**), 5.10 (dd *J*=1.0 and 12.7Hz, 2H, $PhCH_2O$), 5.56-5.65 (m, 3H, C**H**=C**H** and N**H**), 7.30.7.37 (m, 5H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 23.3, 30.8, 35.1, 45.2, 51.9, 52.6, 66.6, 125.1, 125.3, 128.0, 128.5, 136.6, 156.2, 176.6.

FTIR (KBr) 3437, 2951, 1724, 1504 cm^{-1}.

HRMS (EI) 303.1471 (M^+). $C_{17}H_{21}NO_4$ requires 303.1475.

Preparation of (6*R* , 1*R*)-6-(Benzyloxycarbonyl-methyl-amino)-cyclohex-3-ene-carboxylic acid methyl ester (+/-)-5.37.

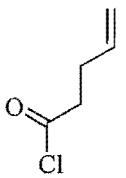


Anhydrous LiCl (26mg, 0.6mmol, 3 equiv), LDA (0.381mL of a 2M solution in THF, 0.47mmol, 2.2 equiv), and MeI (0.053mL, 0.9mmol, 4 equiv) were reacted with a -78° C solution of (+/-)-**5.27** (62mg, 0.21mmol) dissolved in THF (1mL) under nitrogen, according to General Procedure K. Purification by radial chromatography (EA/PE 1:3) gave (+/-)-**5.37** (51mg, 78%) as a colourless oil. Further elution also gave 7mg (11%) of starting material (+/-)-**5.27**.

Reported as a mixture of rotamers: ^{1}H NMR ($CDCl_3$) δ 2.13-2.46 (m, 4H, CH_2CH=$CHCH_2$), 2.83, *2.84* (s, 3H, N**Me**), 2.91 (m, 1H, $CHCO_2Me$), 3.56, *3.58* (s, 3H, O**Me**), 4.39 (m, 1H, NHC**H**), 5.12 (m, 3H, $PhCH_2O$ and N**H**), 5.63 (brs, 2H, C**H**=C**H**), 7.28-7.39 (m, 5H, Ph**H**).

HRMS (ES) 303.1468 (M^+). $C_{17}H_{21}NO_4$ requires 303.1471.

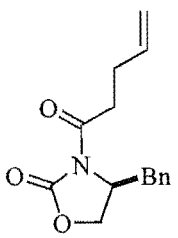
Preparation of 4-Pentenoyl Chloride 5.38.



4-Pentenoic acid (9.5mL, 0.094mol, 1 equiv) was dissolved in dry ether (40mL) and cooled to 0°C. Oxalyl chloride (9.03mL , 0.103mol, 1.1 equiv) was slowly added and the solution stirred at 0°C for 10 minutes. 4 Drops of dry dimethylformamide from a pPasteur pipette were then added and the solution allowed at warm to room temperature overnight. Purification by distillation gave 4-pentenoyl chloride **5.38** as a colourless liquid (10.322g, 93%).

^{1}H NMR ($CDCl_3$) δ 2.45 (q *J*=13.9 and 7.1Hz, 2H, CH_2CH=CH_2), 2.99 (t *J*=7.3Hz, 2H, $ClCOCH_2$), 5.10 (m, 2H, CH=CH_2), 5.78 (m, 1H, C**H**=CH_2).

Preparation of (4*S*)-4-Benzyl-3-pent-4-enoyloxazolidin-2-one (+)-5.39.



(*S*)-(-)-Benzyl-2-oxazolidinone (6g, 33.9mmol, 1 equiv) was dissolved in THF (100mL) and cooled to -78°C under argon. *N*-Butyllithium (24.18mL of 1.6M solution in THF, 37.3mmol, 1.05 equiv) was added dropwise over 10min and the solution was stirred for an additional 10min. 4-Pentenoyl chloride **5.38** (4.23mL, 37.3mmol, 1.05 equiv) was then added dropwise over 10min and the solution stirred at -78°C for 15min and then at rt for

30min. The solution was quenched with sat. aq. NH_4Cl at 0°C, the solvent removed under reduced pressure, and the residue extracted with CH_2Cl_2 (3x50mL). The organic extracts were combined, washed with 1M aqueous NaOH (50mL), water (50mL), brine (50mL), dried ($MgSO_4$) and the solvent removed under reduced pressure to yield (+)-**5.39** (8.593g, 97%) as a colourless oil.

^{1}H NMR ($CDCl_3$) δ 2.45 (ddd *J*=21.5, 8.1 and 1.5Hz, 2H, C**H**$_2$CH=CH$_2$), 2.76 (dd *J*=9.5 and 13.4Hz, 1H, C**H**$_a$Ph), 3..05 (ddt *J*=43.2, 17.4 and 7.3Hz, 2H, NCOC**H**$_2$), 3.29 (dd *J*=13.4 and 3.2Hz, 1H, C**H**$_b$Ph), 4.15 (dd *J*=9.3 and 2.9Hz, 1H, OC**H**$_a$), 4.19 (t *J*=8.1Hz, 1H, OC**H**$_b$), 4.66 (m, 1H, NC**H**), 5.07 (m, 2H, CH=C**H**$_2$), 5.88 (m, 1H, C**H**=CH$_2$), 7.20 (d *J*=7.3Hz, 2H, Ph**H**), 7.27 (t *J*=7.3Hz, 1H, Ph**H**), 7.33 (t *J*=7.3Hz, 2H, Ph**H**).

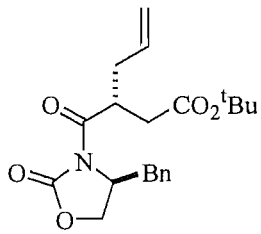
^{13}C NMR ($CDCl_3$) δ 28.1, 34.7, 37.8, 55.1, 66.1, 115.6, 127.3, 128.9, 129.3, 135.2, 136.6, 153.4, 172.5.

FTIR (KBr) 3368, 2924, 1782, 1732, 1643, 1389, 1211cm^{-1}.

HRMS (EI) 259.1213 (M^+). $C_{15}H_{17}NO_3$ requires 259.1208.

$[\alpha]_D$ = +63.4°, c=0.83 $CHCl_3$ (lit.[10] +64.2°).

Preparation of (2*R*,4*S*)-3-((4-(*tert*-Butoxy)-2-allyl-4-oxobutanoyl)-4-phenylmethyl)-1,3-oxazolidin-2-one (+)-5.40a.



NaHMDS (7.381mL of a 1M solution in THF/hexane, 7.38mmol, 1.1 equiv) was added, over 5min, to a -78° C solution of (+)-**5.39a** (1.738g, 6.71mmol, 1 equiv) dissolved in THF (50mL) under argon. and the solution stirred at -78° C for 1h. *tert*-Butyl-bromoacetate (2.971mL, 20.1mmol, 3 equiv) was added and the solution was stirred at -78° C for 3 h. Upon warming to rt, the reaction mixture was partitioned between sat. aq. NH_4Cl (20mL) and ethyl acetate (20mL), and the layers separated. The aqueous layer was back extracted with ethyl acetate (3x10mL) and the combined ethyl acetate extracts dried over $MgSO_4$. Removal of the solvent under reduced pressure, followed by purification by radial

chromatography (EA/PE 15/85), gave (+)-**5.40a** (2.076g, 86%) as a white solid. An analytical sample was obtained by the diffusion of petroleum ether into a solution of (+)-**5.40** dissolved in ethyl acetate. Crystals suitable for X-ray crystallography were obtained (see Appendix for data).

^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H, C($\mathbf{CH_3}$)$_3$), 2.20 (m, 1H, $\mathbf{CH_a}$CH=CH_2), 2.29 (m, 1H, $\mathbf{CH_b}$CH=CH_2), 2.46 (dd *J*=4.1 and 6.8Hz, 1H, $\mathbf{CH_a}CO_2{}^t$Bu), 2.72-2.83 (m, 2H, $\mathbf{CH_a}$Ph and $\mathbf{CH_b}CO_2{}^t$Bu), 3.31 (dd *J*=3.2 and 13.5Hz, 1H, $\mathbf{CH_b}$Ph), 4.13 (dd *J*=3.5 and 8.4Hz, 2H, O$\mathbf{CH_2}$), 4.26 (m, 1H, NCO**CH**), 4.63 (m, 1H, **CH**CH_2Ph), 5.06 (m, 2H, CH=$\mathbf{CH_2}$), 5.77 (m, 1H, **CH**=CH_2), 7.25 (m, 3H, Ph**H**), 7.32 (m, 2H, Ph**H**).

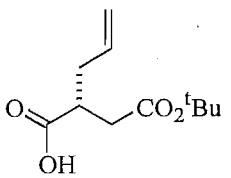
^{13}C NMR δ 28.0, 36.1, 36.6, 37.5, 38.9, 55.4, 65.8, 80.6, 117.7, 127.1, 128.8, 129.4, 134.4, 135.6, 153.0, 171.1, 175.0.

FTIR (KBr) 2978, 1773, 1732, 1699cm^{-1}.

HRMS (ES) 396.1791 (M^++Na). $C_{21}H_{27}NO_5Na$ requires 396.1787.

$[\alpha]_D$ = +51.2°, c=1.0 CH_2Cl_2.

Preparation of (2*R*)-2-Allyl-succinic acid 4-tert-butyl ester (+)-5.41a.



H_2O_2 (1.276mL of 50% w/w in water, 18.8mmol, 4 equiv) and aq. LiOH (225mg, 9.4mmol, 2 equiv, in 20mL of water) were added to a 0°C solution of (+)-**5.40a** (1.75g, 4.7mmol, 1 equiv) in THF (50mL). The reaction was stirred at 0°C for 1 h, whereupon satd. aq. $NaHSO_3$ (10mL), and satd. aq. $NaHCO_3$ (10mL) were added and the mixture stirred for an additional 20min. THF was removed under reduced pressure and the residue diluted with CH_2Cl_2 (10mL) and water (10mL). The organic layer was separated, the solvents removed under reduced pressure, and the resulting white solid recrystallised from EA/PE to yield (*S*)-(-)-benzyl-2-oxazolidinone (821mg, 99%) as white needles. The aqueous layer was then acidified with 3M aqueous HCl and extracted with CH_2Cl_2 (3x10mL). The combined organic extracts were washed with water (10mL), brine (10mL),

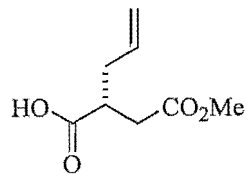
and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield (+)-**5.41a** (953mg, 95%) as a white solid.

^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H, C(**CH$_3$**)$_3$), 2.30 (m, 1H, **CH$_a$**CH=CH$_2$), 2.39-2.48 (m, 2H, **CH$_b$**CH=CH$_2$ and **CH$_a$**CO$_2$^{t}Bu), 2.59 (dd *J*=16.8 and 8.9Hz, 1H, **CH$_b$**CO$_2$^{t}Bu), 2.90 (m, 1H, HO$_2$CC**H**), 5.09 (m, 2H, CH=C**H$_2$**), 5.74 (m, 1H, C**H**=CH$_2$).

^{13}C NMR δ 28.0, 35.6, 36.2, 40.9, 81.0, 118.0, 134.2, 171.0, 180.3.

$[\alpha]_D$ = +3.4°, c=1.4 CH_2Cl_2. (lit.[11] +3.4°)

Preparation of (2*R*)-2-Allyl-succinic acid 4-methyl ester (-)-5.41b.

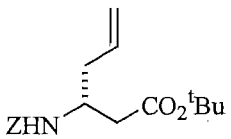


H_2O_2 (0.168mL of 50% w/w in water, 2.48mmol, 4 equiv) and aq. LiOH (29mg, 1.24mmol, 2 equiv, in 3mL of water) were added to a 0°C solution of **5.40b** (205mg, 0.62mmol, 1 equiv) in THF (10mL). The reaction was stirred at 0°C for 1 h, whereupon satd. aq. $NaHSO_3$ (10mL), and satd. aq. $NaHCO_3$ (10mL) were added and the mixture stirred for an additional 20min. THF was removed under reduced pressure and the residue diluted with CH_2Cl_2 (10mL) and water (10mL). The organic layer was separated, the solvents removed under reduced pressure, and the resulting white solid recrystallised from EA/PE to yield (*S*)-(-)-benzyl-2-oxazolidinone (106mg, 97%) as white needles. The aqueous layer was then acidified with 3M aqueous HCl and extracted with CH_2Cl_2 (3x10mL). The combined organic extracts were washed with water (10mL), brine (10mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield (-)-**5.41b** (54mg, 51%) as a white solid.

^{1}H NMR ($CDCl_3$) δ 2.34 (m, 1H, **CH$_a$**CH=CH$_2$), 2.47 (m, 2H, **CH$_b$**CH=CH$_2$ and **CH$_a$**CO$_2$Me), 2.69 (dd *J*=8.9 and 16.8Hz, 1H, **CH$_b$**CO$_2$Me), 2.97 (m, 1H, HO$_2$CC**H**), 3.68 (s, 3H, O**Me**), 5.10 (m, 2H, CH=C**H$_2$**), 5.73 (m, 1H, C**H**=CH$_2$).

$[\alpha]_D$ c=-2.4° (0.5 CH_2Cl_2)

Preparation of *tert*-Butyl (3*R*)-*N*-(Benzyloxycarbonyl)-3-amino–4-allylbutanoate (+)-5.42a.



Et_3N (1.073mL, 7.71mmol, 1.1 equiv), DPPA (1.508mL, 7.01mmol, 1 equiv), and benzyl alcohol (2.175mL, 21.03mmol, 3 equiv) were reacted with a solution of (+)-**5.41a** (1.5g, 7.01mmol, 1 equiv) in toluene (60mL), according to Modified General Procedure M. Purification by column chromatography (EA/PE 1:9) gave (+)-**5.42a** (1.752g, 79%) as a colourless oil.

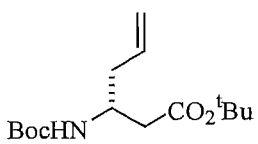
^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H, C(C**H**$_3$)$_3$), 2.32 (m, 2H, C**H**$_2$CH=CH$_2$), 2.42 (d *J*=5.4Hz, 2H, C**H**$_2$CO$_2$^{t}Bu), 4.03 (m, 1H, NC**H**), 5.08 (m, 4H, CH=C**H**$_2$ and PhC**H**$_2$), 5.27 (d *J*=8.3Hz, 1H, N**H**), 5.76 (m, 1H, C**H**=CH$_2$), 7.28 (m, 5H, Ph**H**).. Compare series 6 of Evans ref

^{13}C NMR δ 27.9, 38.7, 39.3, 47.7, 66.5, 81.0, 115.3, 118.2, 127.9, 1128.4, 133.9, 136.5, 155.6, 170.7.

HRMS (ES) 342.1680 (M^++Na). $C_{18}H_{25}NO_4Na$ requires 342.1681.

$[\alpha]_D$ = +1.8°, c=1.0 CH_2Cl_2.

Preparation of (3*R*)-3-*tert*-Butoxycarbonylamino-hex-5-enoic acid tert-butyl ester (-)-5.43.

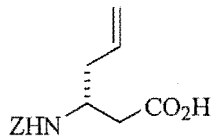


Et_3N (0.078mL, 0.56mmol, 1.2 equiv), $ClCO_2Et$ (0.050mL, 0.52mmol, 1.1 equiv), NaN_3 (76mg in 1mL of water, 1.17mmol, 2.5 equiv), and *tert*-butyl alcohol (0.134mL, 1.4mmol, 3 equiv) were reacted with a solution of (+)-**5.41a** (100mg, 0.47mmol), dissolved in dry acetone (5mL), according to General Procedure M. Purification by column chromatography (EA/PE 1:9) gave (-)-**5.43** (90mg, 68%) as a colourless oil.

^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H, C(CH_3)$_3$), 1.44 (s, 9H, C(CH_3)$_3$), 2.28 (m, 2H, $CH_2CH{=}CH_2$), 2.41 (d *J*=5.4Hz, 2H, $CH_2CO_2{}^tBu$), 3.95 (m, 1H, NC**H**), 4.99 (s, 1H, N**H**), 5.08 (m, 2H, CH=CH_2), 5.76 (m, 1H, C**H**=CH_2).

$[\alpha]_D = -9.8°$, c=1.1 MeOH, (lit.[11] -10.09°).

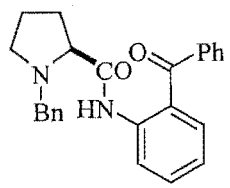
Preparation of (3*R*)-3-Benzyloxycarbonylamino-hex-5-enoic acid 5.44.



Me_2S (0.346mL, 4.7mmol, 10 equiv) was added to a 0° C solution of (+)-**5.42** (150mg, 0.47mmol, 1 equiv), dissolved in CH_2Cl_2/TFA (7:3, 4mL), and the mixture stirred at rt for 3.5h. Upon removal of the solvents under reduced pressure, the residue was taken up in CH_2Cl_2 (5mL) and extracted with 1M NaOH (15mL). The aqueous layer was then washed with CH_2Cl_2 (2x15mL), acidified to pH 1 with 1M HCl, and extracted with CH_2Cl_2 (3x10mL). The combined organic extracts were dried ($MgSO_4$), and the solvent removed under reduced pressure to give **5.44** (107mg, 87%) as a colourless oil.

^{1}H NMR ($CDCl_3$) δ 2.36 (m, 2H, CH_2CH=CH_2), 2.61 (d *J*=4.9Hz, 2H, CH_2CO_2H), 4.08 (m, 1H, NC**H**), 5.11 (m, 4H, CH=CH_2 and PhCH_2), 5.21 (d *J*=7.8Hz, 1H, N**H**), 5.74 (m, 1H, C**H**=CH_2), 7.34 (m, 5H, Ph**H**).

Preparation of (*S*)-2-[N-(N'-benzylprolyl)-amino]benzophenone (BPB) 5.47.



Freshly distilled $SOCl_2$ (0.074mL, 1.02mmol, 2 equiv) was slowly added to a solution of (*S*)-*N*-benzyl proline (166mg, 0.81mmol, 1.6 equiv) suspended in CH_2Cl_2 (5mL) at -30° C, and the mixture stirred at -30° C until it became semi-transparent. A solution of 2-aminobenzophenone (100mg, 0.51mmol, 1 equiv) in CH_2Cl_2 (2mL) was then added and the solution stirred at -30° C for 10h. After warming to 0° C, a solution of Na_2CO_3

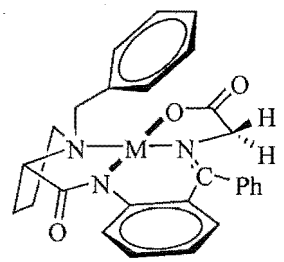
(161mg, 1.52mmol, 3 equiv, in 3mL of water) was added and the organic layer separated. The aqueous layer was extracted with CH_2Cl_2 (3x10mL) and the combined organic extracts dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue purified by radial chromatography (acetone/$CHCl_3$ 1:9, streaky) to give **5.47** (113mg, 58%) as a white solid.

mp 99-101° C, (lit.[12] 101-102°).

^{1}H NMR (CDOD 500MHz) δ 1.80 (m, 2H), 1.96 (m, 1H), 2.25 (ddd *J*=22.7, 13.2 and 9.8Hz, 1H), 2.41 (dd *J*=9.6 and 16.4Hz, 1H), 3.22 (t *J*=6.8Hz, 1H, α-**H**pro), 3.32 (dd *J*=4.8 and 14.8Hz, 1H), 3.59 (d *J*=13.2Hz, 1H, C**H**$_a$Ph), 3.92 (d *J*=13.2Hz, 1H, C**H**$_b$Ph), 7.07-8.56 (m, 14H, Ph**H**), 11.53 (brs (1H, N**H**).

Note: Commercial samples of BPB were used in all subsequent reactions.

Preparation of Gly-Ni-BPB Complex (+)-5.48.[12]



A solution of 1.2N MeONa (15mL, 18mmol, 5 equiv) was quickly added to a 50° C suspension of BPB **5.47** (1g, 2.6mmol, 1 equiv, commercial sample), $Ni(NO_3)_2.6H_2$) (1.49g, 5.1mmol, 2 equiv), and glycine (0.97g, 13mmol, 4 equiv) dissolved in MeOH (50mL) under argon, and the resulting red mixture was stirred vigorously at 50° C for 2h. Water (50mL) was then added and the complex extracted with $CHCl_3$ (4x20mL). The combined organic extracts were dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by column chromatography ($CHCl_3$/acetone 5:1) gave (+)-**5.48** (1.158g, 90%) as a red solid.

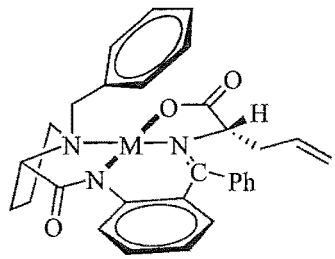
^{1}H NMR (CD_3OD) δ 1.75 (m, 3H, prolyl-**H**), 2.23 (m, 1H, prolyl-**H**), 2.52 (m, 1H, prolyl-**H**), 3.19 (m, 1H, prolyl-**H**), 2.29 (m, 1H, prolyl-**H**), 3.30 (t *J*=1.4Hz, 2H, gly-C**H**$_2$), 3.73 (dd *J*=12.7 and 32.2Hz, 2H, PhC**H**$_2$), 7.09-8.18 (14H, Ph**H**).

LRMS (ES) 498.0 (M^+-H). $C_{27}H_{26}N_3O_3Ni$ requires 499.2.

$[\alpha]_D$ = +1880 (c=0.125*, MeOH), (lit.[13] +2006°, c=1.0 MeOH).

*Unable to obtain a reading at c=1.0, MeOH.

Preparation Alkylation of Gly-Ni-BPB Complex with Allyl Bromide of 5.49.[14]

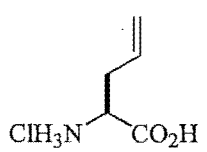


Finely powdered NaOH (200mg, 5mmol, 2.5 equiv) and allyl bromide (0.26mL, 3mmol, 1.5 equiv) were added to a stirred mixture of the red Ni-BPB-glycine complex (+)-**5.48** (1g, 2mmol, 1 equiv), in dry CH_3CN (10mL) under N_2, and the mixture was stirred at rt for 3h. 0.1M HCl (30mL) was then added and the solution extracted with CH_2Cl_2 (4x20mL). The organic extracts were combined, dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification by column chromatography (2:1 CH_2Cl_2/Me_2CO) gave **5.49** (1.02g, 93%) as a red solid.

^{1}H NMR ($CDCDCl_3$ 500MHz) δ 2.0-2.17 (m, 2H), 2.38-2.59 (m, 3H), 2.77 (m, 1H), 3.41-3.54 (m, 3H), 3.59 (d *J*=12.5Hz, 1H, C**H**$_a$Ph), 4.02 (m, 1H, gly-**αH**), 4.42 (d *J*=12.5Hz , 1H, C**H**$_b$Ph), 5.15-5.41 (m, 2H, CH=C**H**$_2$), 6.43 (m, 1H, C**H**=CH_2), 6.61-8.17 (m, 14H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 23.3 (γ-**C**pro), 30.7 (β-**C**pro), 38.4 (**C**H_2CH=CH_2), 56.8 (δ-**C**pro), 63.1 (**C**H_2Ph), 70.2, 70.3 (α-**C**pro and **C**HCH$_2$CH=CH_2), 119.7 (CH=**C**H_2), 120.65 (Ar**C**), 123.6 (**C**H=CH_2), 126.4, 127.0, 127.7, 128.5, 128.8, 129.0, 129.7, 130.0, 131.5, 132.1, 132.2, 133.1, 133.3, 133.9, 142.4 (Ar**C**), 170.8 (**C**=N), 178.8 (N**C**O), 180.3 (CH**C**O_2).

Preparation of Allylglycine.HCl (-)-5.50.



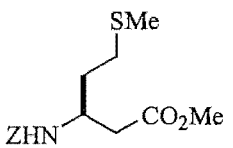
A solution of (+)-**5.49** (1.02g, 1.9mmol) in MeOH (33mL) was added to a warm 2N HCl solution (23mL) and the mixture stirred at reflux for 1h. The resulting pale orange solution was then cooled to rt and conc. NH_4 added until the pH was between 9-10. The pale green aqueous layer was extracted with CH_2Cl_2 (3x20mL), the organic extracts combined, and the solvent removed under reduced pressure to give BPB (+)-**5.47** (681mg, 97%) as a white solid. The remaining blue aqueous layer was purifed by ion exchange chromatography (1N HCl) to give (-)-**5.50** (234mg, 82%), as a white solid.

^{1}H NMR (D_2O) δ 2.53-2.68 (m, 2H, C**H**$_2$CH=CH$_2$), 3.92 (m, 1H, **αH**), 5.31 (m, 2H, CH=C**H**$_2$), 5.69 (m, 1H, C**H**=CH$_2$).

$[\alpha]_D$ = -6.2° c=2.1, 6N HCl, (lit.[14] 6.4°).

8.7 Experimental Described in Chapter Six

Preparation of (3*R*)-3-Benzyloxycarbonylamino-5-methylsulfanyl-pentanoic acid methyl ester (-)-6.16.



Et_3N (2.548mL, 17.67mmol, 1 equiv), $ClCO_2Et$ (1.663mL, 17.67mmol, 1 equiv) and ethereal diazomethane, were reacted with a -15° C solution of (*S*)-*N*-Cbz-methionine **6.15** (5g, 17.67mmol), in THF (90mL) under nitrogen, according to General Procedure Ja. The resulting diazoketone was dissolved in dry MeOH (70mL) and reacted with silver benzoate (445mg, 1.94mmol, 0.11 equiv) dissolved in Et_3N (7.128mL, 51.24mmol, 2.9 equiv), according to General Procedure Jb. Purification by column chromatography (EA/PE 1:3) gave (-)-**6.16** (5.12g, 93%) as a colourless oil.

^{1}H NMR ($CDCl_3$) δ 1.80 (m, 1H, C**H**$_a$CH$_2$SMe), 1.88 (m, 1H, C**H**$_b$CH$_2$SMe), 2.09 (s, 3H, S**Me**), 2.52 (m, 2H, C**H**$_2$SMe), 2.58 (m, 2H, C**H**$_2$CO$_2$Me), 3.66 (s, 3H, O**Me**), 4.09 (m, 1H, NHC**H**), 5.08 (s, 2H, PhC**H**$_2$), 5.35 (d *J*=8.8Hz, 1H, N**H**), 7.32 (m, 5H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 15.4, 30.6, 33.6, 38.5, 47.3, 51.7, 66.6, 128.0, 128.0, 128.4, 136.4, 155.7, 171.7.

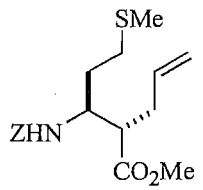
FTIR (KBr) 3321, 1736, 1690, 1541cm^{-1}.

HRMS (ES) 312.1272 (M^++H). $C_{15}H_{22}NO_4S$ requires 312.1270.

Micro. Cald for $C_{15}H_{21}NO_4S$. C, 57.81; H, 6.80; N, 4.50; S, 10.50. Found: C, 57.61; H, 7.04; N, 4.52; S, 10.54.

$[\alpha]_D$=-18.8° c=1.0 $CHCl_3$

Preparation of (*2S,1S*)-*2*-(1-Benzyloxycarbonylamino-3-methylsulfanyl-propyl)-pentanoic acid methyl ester (-)-6.17.



Anhydrous LiCl (198mg, 4.8mmol, 3 equiv), LDA (1.768mL of a 2M solution in THF, 3.54mmol, 2.2 equiv), and allyl bromide (0.557mL, 6.43mmol, 4 equiv) were reacted with a -78° C solution of (-)-**6.16** (500mg, 1.61mmol, 1 equiv) dissolved in THF (10mL) under nitrogen, according to General Procedure K. Purification by radial chromatography (EA/PE 1:4) gave (-)-**6.17** (301mg, 53%) as a colourless oil.

^{1}H NMR ($CDCl_3$) δ 1.72 (m, 2H, CH_2CH_2SMe), 2.08 (s, 3H, SMe), 2.31 (m, 1H, C**H**$_a$CH=CH_2), 2.41 (m, 1H, C**H**$_b$CH=CH_2), 2.52 (m, 2H, CH_2SMe), 2.66 (m, 1H, C**H**CO_2Me), 3.67 (s, 3H, O**Me**), 3.99 (m, 1H, NHC**H**), 5.03-5.14 (m, 4H, CH=C**H**$_2$ and PhC**H**$_2$), 5.57 (d *J*=9.8Hz, 1H, NH), 5.74 (m, 1H, C**H**=CH_2), 7.32-7.37 (m, 5H, Ph**H**).

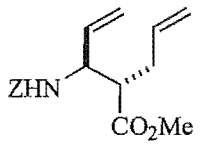
^{13}C NMR ($CDCl_3$) δ 15.3, 30.4, 33.8, 33.8, 48.2, 50.7, 51.4, 66.4, 117.3, 127.8, 127.8, 128.2, 134.2, 136.3, 156.1, 174.3.

FTIR (KBr) 3342, 2953, 1717, 1701, 1653, 1506cm^{-1}.

HRMS (EI) 351.1516 (M^+). $C_{18}H_{25}NO_4S$ requires 351.1504.

$[\alpha]_D$ = -20.9°, c=1.0 $CHCl_3$

Preparation of (*2S,3S*)-*2*-Allyl-3-benzyloxycarbonylamino-2-methyl-pent-4-enoic acid methyl ester (-)-6.18.



A: Alkene (-)-**6.17** (295mg, 0.84mmol, 1 equiv) was dissolved in acetic acid (2mL) and treated with H_2O_2 (0.086mL of a 50% w/w solution, 1.26mmol, 1.4 equiv) according to General Procedure Ia. The resulting sulfoxide was then dissolved in degassed *m*-xylene (10mL) and underwent oxidative elimination according to General Procedure Ib. Purification by radial chromatography (EA/PE 1:3) gave diene (-)-**6.18** (211mg, 76%) as a yellow oil.

B: Anhydrous LiCl (94mg, 2.3mmol, 3 equiv), LDA (0.837mL of a 2M solution in THF, 1.67mmol, 2.2 equiv), and allyl bromide (0.263mL, 3.0mmol, 4 equiv) were reacted with a -78° C solution of **6.22** (200mg, 0.76mmol, 1 equiv) dissolved in THF (4mL) under nitrogen, according to General Procedure K. Purification by radial chromatography (EA/PE 15:85) gave (-)-**6.18** (62mg, 27%) as a yellow oil.

^{1}H NMR ($CDCl_3$) δ 2.35 (m, 1H, C**H**$_a$CH=CH_2), 2.43 (m, 1H, C**H**$_b$CH=CH_2), 2.72 (m, 1H, C**H**CO_2Me), 3.63 (s, 3H, O**Me**), 4.45 (m, 1H, NHC**H**), 5.05-5.23 (m, 6H, 2xCH=C**H**$_2$ and PhC**H**$_2$), 5.76 (m, 2H, 2xC**H**=CH_2), 7.32-7.37 (m, 5H, Ph**H**).

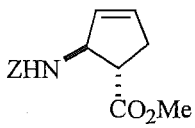
^{13}C NMR ($CDCl_3$) δ 33.8, 48.6, 51.5, 53.4, 66.7, 115.8, 117.6, 127.9, 128.0, 128.4, 134.2, 136.3, 136.4, 155.9, 174.1.

FTIR (KBr) 3342, 2953, 1724, 1643, 1504cm^{-1}.

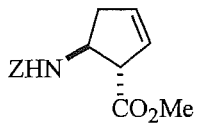
HRMS (EI) 303.1465 (M^+). $C_{17}H_{21}NO_4$ requires 303.1471.

$[\alpha]_D$ = -37.1°, c=1.0 $CHCl_3$.

Preparation of (*3S,2S*)-*3*-Benzyloxycarbonylamino-cyclopent-3-ene carboxylic acid methyl ester (-)-6.19, and (*5S,1S*)-*5*-Benzyloxycarbonylamino-cyclopenten-2-enecarboxylic acid methyl ester 6.23.



(+)-6.19



6.23

Catalyst **1.42** (4.2mg,, 5mol%), dissolved in *dry degassed* benzene (0.5mL), was added to a solution of diene (-)-**6.18** (30mg, 0.1mmol, 1 equiv), dissolved in *dry degassed* benzene (1mL) under argon, according to General Procedure E. The mixture was then stirred at reflux for 16h. Attempted purification by radial chromatography (EA/PE 1:3) gave a fraction containing **6.19** and **6.23** (24mg, 89%), in a ratio of 1.5:1 by ^{1}H NMR, that could not be separated further.

In a second reaction, catalyst **1.42** (18mg,, 5mol%), dissolved in *dry degassed* benzene (0.5mL), was added to a solution of diene (-)-**6.18** (126mg, 0.42mmol, 1 equiv), dissolved in *dry degassed* benzene (4mL) under argon, according to General Procedure E. The mixture was then stirred at rt for 2h. Purification by radial chromatography (EA/PE 1:3) gave (+)-**6.19** (105mg, 92%) as a white solid. An analytical sample was obtained by the diffusion of petroleum ether into a solution of **6.19** dissolved in ethyl acetate.

mp 92-93° C

^{1}H NMR ($CDCl_3$) δ 2.62 (m, 1H, CH=CHC**H**$_a$), 2.75 (m, 1H, C**H**CO_2Me), 2.87 (m, 1H, CH=CHC**H**$_b$), 3.72 (s, 3H, O**Me**), 4.86 (brs, 1H, N**H**), 5.06 (m, 1H, NHC**H**), 5.12 (m, 2H, Ph**C**H_2), 5.63 (brs, 1H, CH=C**H**CH_2), 5.86 (m, 1H, C**H**=$CHCH_2$), 7.30-7.38 (m, 5H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 35.5, 50.7, 52.1, 61.1, 66.7, 128.1, 128.5, 130.0, 132.4, 136.3, 155.5, 174.8.

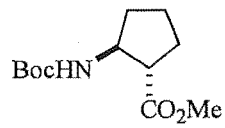
FTIR (KBr) 2953, 1734, 1684, 1537cm^{-1}.

HRMS (EI) Found 275.1158 (M^+). $C_{15}H_{17}NO_4$ requires 275.1158.

Micro. Calcd for $C_{15}H_{17}NO_4$. C, 65.44; H, 6.23; N, 5.09. Found: C, 65.13; H, 6.31; N, 5.25.

$[\alpha]_D$ = +102.3°, c=1.0 $CHCl_3$.

Preparation of (*3S,2S*)-3-*tert*-Butyloxycarbonylamino-cyclopentane carboxylic acid methyl ester (+)-6.20.

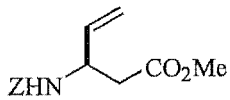


10%-Palladium-on-carbon (6mg, 20% w/w), $NaHCO_3$ (14mg, 0.17mmol, 1.5 equiv), and di-*tert*-butyl-dicarbonate (37mg, 0.17mmol, 1.5 equiv) were reacted with (+)-**6.19** (30mg, 0.11mmol), dissolved in dry MeOH (3mL) under a hydrogen atmosphere, according to Modified General Procedure L. Purification by radial chromatography (EA/PE 1:7) gave (+)-**6.20** (20mg, 75%) as a white solid.

^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H, C(C**H**$_3$)$_3$), 1.47 (m, 1H, NHCHC**H**$_a$) 1.72 (m, 2H, C**H**$_a$CHCO$_2$Me and C**H**$_a$CH$_2$CHCO$_2$Me), 1.89 (m, 1H, C**H**$_b$CH$_2$CHCO$_2$Me), 1.97 (m, 1H, C**H**$_b$CHCO$_2$Me), 2.11 (m, 1H, NHCHC**H**$_b$), 2.57 (dd *J*=16.1 and 8.3Hz, 1H, C**H**CO$_2$Me), 3.68 (s, 3H, O**Me**), 4.11 (m, 1H, NHC**H**), 4.57 (brs, 1H, N**H**).

$[\alpha]_D$ = +41.6°, c=0.65 $CHCl_3$. (lit.[15] +44.6°, c=1.3).

Preparation of (3*S*)-3-Benzyloxycarbonylamino-pentenoic acid methyl ester 6.22.



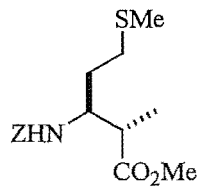
Methyl ester (-)-**6.16** (720mg, 2.32mmol)) was dissolved in acetic acid (5mL) and treated with H_2O_2 (0.230mL of a 50% w/w solution, 3.25mmol, 1.4 equiv) according to General Procedure Ia. The resulting sulfoxide was then dissolved in degassed *m*-xylene (10mL) and underwent oxidative elimination according to General Procedure Ib. Purification by radial chromatography (EA/PE 15:85) gave **6.22** (430mg, 63%) as a yellow oil.

^{1}H NMR ($CDCl_3$) δ 2.65 (m, 2H, C**H**$_2$CO$_2$Me), 3.67 (s, 3H, O**Me**), 4.58 (m, 1H, NHC**H**), 5.11 (s, 2H, PhC**H**$_2$O), 5.18 (m, 2H, CH=C**H**$_2$), 5.45 (brs, 1H, N**H**), 5.85 (m, 1H, C**H**=CH$_2$), 7.31-7.45 (m, 5H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 38.5, 49.5, 51.3, 66.2, 67.4, 115.15, 127.6, 127.9, 128.0, 136.4, 136.5, 155.4, 171.0.

FTIR (KBr) 3339, 2953, 1720.4, 1514cm^{-1}.

Preparation of (*3S,2S*)-3-Benzyloxycarbonylamino-2-methyl-5-methylsulfanyl-pentanoic acid methyl ester (-)-6.24.



Anhydrous LiCl (396mg, 9.65mmol, 3 equiv), LDA (3.537mL of a 2M solution in THF, 7.07mmol, 2.2 equiv), and MeI (0.801mL, 12.86mmol, 4 equiv) were reacted with a -78° C solution of (-)-**6.16**. (1g, 3.22mmol), dissolved in THF (15mL) under nitrogen, according to General Procedure K. Purification by radial chromatography (EA/PE 1:4) gave (-)-**6.24** (0.962g (92%) as a colourless oil.

^{1}H NMR ($CDCl_3$) δ 1.22 (d *J*=6.8Hz, 3H, CHC**H$_3$**), 1.73 (m, 2H, C**H$_2$**CH$_2$SMe), 2.08 (s, 3H, S**Me**), 2.52 (m, 2H, C**H$_2$**SMe), 2.70 (m, 1H, C**H**CO$_2$Me), 3.67 (s, 3H, O**Me**), 3.91 (m, 1H, NHC**H**), 5.10 (dd *J*=12.4 and 14.4Hz, 2H, PhC**H$_2$**), 5.49 (d *J*=9.8Hz, 1H, N**H**), 7.29-7.37 (m, 5H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 14.8, 15.5, 30.7, 33.6, 42.6, 51.7, 52.6, 66.6, 127.9, 128.0, 128.4, 136.5, 156.5, 175.4.

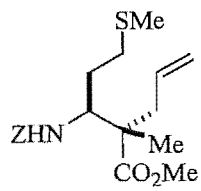
FTIR (KBr) 3337, 2953, 1717, 1699, 1510, 1454cm^{-1}.

HRMS (ES) 326.1429 (M^++H). $C_{16}H_{24}NO_4S$ requires 326.1426.

$[\alpha]_D$ = -14.3°, c=1.0 $CHCl_3$.

Micro. Calcd for C:59.05, H:7.12, N:4.30, S:9.85; Found C:59.15, H:7.30, N:4.40, S:9.95.

Preparation of (*2S,1S*)-*2*-(1-Benzyloxycarbonyl-3-methylsulfanyl-propyl)-2-methyl-pent-3-enoic acid methyl ester (-)-6.25.



Anhydrous LiCl (326mg, 7.95mmol, 3 equiv), LDA (2.918mL of a 2M solution in THF, 5.83mmol, 2.2 equiv), and allyl bromide (0.918mL, 10.6mmol, 4 equiv) were reacted with a -78° C solution of (-)-**6.24** (862mg, 2.65mmol), dissolved in THF (15mL) under nitrogen,

according to General Procedure K. Purification by radial chromatography (EA/PE 1:9) gave (-)-**6.25** (411mg, 43%) as a colourless oil.

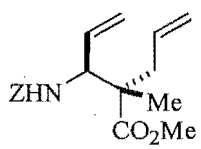
^{1}H NMR ($CDCl_3$) δ 1.19 (s, 3H, CHC**H$_3$**), 1.42 (m, 1H, C**H$_a$**CH$_2$SMe), 1.91 (m, 1H, C**H$_b$**CH$_2$SMe), 2.07 (s, 3H, S**Me**), 2.23 (dd *J*=7.4 and 13.7Hz, 1H, C**H$_a$**CH=CH$_2$) 2.43-2.58 (m, 3H, C**H$_b$**CH=CH$_2$ and C**H$_2$**SMe), 3.66 (s, 3H, O**Me**), 3.79 (dt *J*=10.7 and 2.4Hz, 1H, NHC**H**), 5.02-5.16 (m, 4H, CH=C**H$_2$** and PhC**H$_2$**O), 5.42 (d *J*=10.7Hz, 1H, N**H**), 5.67 (m, 1H, C**H**=CH$_2$), 7.31-7.37 (m, 5H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 15.7, 19.5, 31.2, 31.4, 41.2, 49.8, 51.8, 56.3, 66.7, 118.9, 128.0, 128.1, 128.4, 132.9, 136.5, 156.4, 175.8.

FTIR (KBr) 3343, 2951, 1720, 1641, 1510cm^{-1}.

$[\alpha]_D$ = -30.6°, c=1.0 $CHCl_3$.

Preparation of (2*S*,3*S*)-2-Allyl-3-benzyloxycarbonylamino-2-methyl-pent-4-enoic acid methyl ester (-)-6.26.



Alkene (-)-**6.25** (352mg, 0.97mmol) was dissolved in acetic acid (2mL) and treated with H_2O_2 (0.092mL of a 50% w/w solution, 1.35mmol, 1.4 equiv) according to General Procedure Ia. The resulting sulfoxide was then dissolved in degassed *m*-xylene (10mL) and underwent oxidative elimination according to General Procedure Ib. Purification by radial chromatography (EA/PE 1:3) gave diene (-)-**6.26** (267mg, 88%) as a colourless oil

^{1}H NMR ($CDCl_3$) δ 1.17 (s, 3H, CC**H$_3$**), 2.26 (dd *J*=7.3 and 13.7Hz, 1H, C**H$_a$**CH=CH$_2$), 2.51 (dd *J*=7.3 and 13.7Hz, 1H, C**H$_b$**CH=CH$_2$), 3.65 (s, 3H, O**Me**), 4.22 (t *J*= 8.3Hz, 1H, NHC**H**), 5.04-5.26 (m, 6H, PhC**H$_2$**O and 2xCH=C**H$_2$**), 5.71 (m, 2H, 2xC**H**=CH$_2$), 7.26-7.36 (m, 5H, Ph**H**).

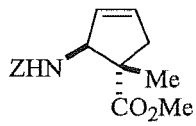
^{13}C NMR ($CDCl_3$) δ 19.1, 41.0, 49.3, 51.8, 59.2, 66.7, 117.83, 119.0, 128.1, 128.4, 132.7, 134.3, 136.4, 155.7, 175.6.

FTIR (KBr) 3342, 2951, 1707, 1641, 1501cm^{-1}.

HRMS (ES) 340.1526 (M^++Na). $C_{18}H_{23}NO_4Na$ requires 340.1525.

$[\alpha]_D$= -30.2, c=1.0 $CHCl_3$

Preparation of (1*S*,2*S*)-2-Benzyloxycarbonylamino-1-methyl-cyclopen-3-ene-carboxylic acid methyl ester (+)-6.27.



Catalyst **1.42** (24mg,, 5mol%), dissolved in *dry degassed* benzene (0.5mL), was added to a solution of diene (-)-**6.26** (180mg, 0.57mmol), dissolved in *dry degassed* benzene (5mL) under argon, according to General Procedure E. The mixture was then stirred at rt for 16h. Purification by radial chromatography (EA/PE 1:3) gave (+)-**6.27** (151mg, 92%) as a white solid.

^{1}H NMR ($CDCl_3$) δ 2.24 (d *J*=17.1Hz, 1H, CH=CHC**H**$_a$), 2.95 (d *J*=17.1Hz, 1H, CH=CHC**H**$_b$), 3.73 (s, 3H, O**Me**), 4.74 (brd *J*=7.3Hz, 1H, N**H**), 5.10 (dd$_{AB}$ *J*=12.5Hz, 2H, PhC**H**$_2$), 5.21 (brd *J*=9.3Hz, 1H, NHC**H**), 5.53 (brs, 1H, CH=C**H**CH$_2$), 5.83 (brs, 1H, C**H**=CHCH$_2$), 7.25-7.36 (m, 5H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 18.8, 44.2, 51.4, 52.1, 62.8, 66.5, 127.9, 128.3, 129.5, 131.6, 131.8, 136.4, 155.7, 177.2.

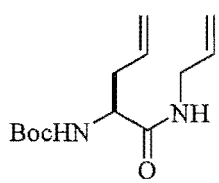
FTIR (KBr) 3350, 2951, 1697, 1634, 1502cm^{-1}.

HRMS (ES) 290.1396 (M^++H). $C_{16}H_{19}NO_4$ requires 290.1392.

$[\alpha]_D$ = +50.5°, c=1.0 $CHCl_3$.

8.8 Experimental Described in Chapter Seven

Preparation of (1-Allylcarbamoyl-but-3-enyl)-carbamic acid *tert*-butyl ester (+/-)-7.24.



EDCI (459mg, 2.24mmol, 1.3 equiv), HOBt (378mg, 2.58mmol, 1.5 equiv), allylamine (0.145mL, 2.58mmol, 1.5 equiv) and diisopropylethylamine (0.415mL, 1.9mmol, 1.1 equiv) were added to a solution of *N*-Boc (+/-)-allyl glycine **7.23** (370mg, 1.72mmol, 1

equiv) dissolved in CH_2Cl_2 (15mL) under argon. The mixture was stirred at rt under argon for 16h. The solvent was removed under reduced pressure and the residue purified by radial chromatography (EA/PE 1:3) to give (+/-)-**7.24** (404mg, 92%) as a white solid.

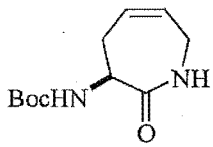
^{1}H NMR ($CDCL_3$) δ 1.42 (s, 9H, CC**H**$_3$), 2.48 (m, 2H, CHC**H**$_2$CH=CH$_2$), 3.85 (m, 2H, NHC**H**$_2$CH=CH$_2$), 4.18 (bs, 1H, **αH**), 5.14 (m, 5H, 2xCH=C**H**$_2$ and N**H**CH), 5.76 (m, 2H, 2xC**H**=CH2), 6.53 (bs, 1H, N**H**CH$_2$).

^{13}C NMR δ 28.2, 36.9, 41.7, 53.8, 80.1, 116.2, 118.8, 133.1, 133.8, 155.6, 171.3.

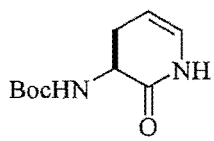
FTIR (KBr) 3261, 2980, 1697, 1661, 1547cm^{-1}

HRMS (ES) 277.1517 (M^++Na). $C_{13}H_{22}N_2O_3Na$ requires 277.1528.

Preparation of (2-Oxo-2,3,4,7-tetrahydro-1H-azepin-3-yl)-carbamic acid *tert*-butyl ester (+/-)-7.25 and (2-Oxo-1,2,3,4-tetrahydro-pyridin-3-yl)-carbamic acid *tert*-butyl ester (+/-)-7.26.



7.25



7.26

Catalyst **1.42** (9mg, 5mol%), dissolved in *dry degassed* benzene (0.5mL), was added to a solution of diene (+/-)-**7.24** (60mg, 0.24mmol), dissolved in *dry degassed* benzene (5mL) under argon, according to General Procedure E. The solution was then refluxed vigorously for 4h. ^{1}H NMR analysis of the crude revealed it to contain a 1:1 mixture of (+/-)-**7.25** and (+/-)-**7.26**. Purification by radial chromatography (EA/PE 1:3) gave (+/-)-**7.25** (23mg, 46%) as a white solid. Further elution gave (+/-)-**7.26** (24mg, 45%) as a white solid.

In a second reaction, catalyst **1.42** (8mg, 5mol%), dissolved in *dry degassed* benzene (0.5mL), was added to a solution of diene (-)-**6.26** (50mg, 0.2mmol), dissolved in *dry degassed* benzene (4mL) under argon, according to General Procedure E. The mixture was then refluxed at 85° C for 2h. ^{1}H NMR analysis of the crude showed the exclusive formation of (+/-)-**7.25**. Purification by radial chromatography (EA/PE 1:1) gave (+/-)-**7.25** (40mg, 90%) as a white solid.

Data for (+/-)-**7.25**: ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H, C(CH$_3$)$_3$), 2.25 (m, 1H, CHC**H**$_a$CH=CH), 2.65 (bd *J*=18Hz, 1H, CHC**H**$_b$CH=CH), 3.43 (m, 1H, NHCH$_a$), 4.15 (bd

J=7.1Hz, 1H, NHC**H**$_b$), 4.82 (m, 1H, NHC**H**), 5.68 (m, 1H, =C**H**CH$_2$NH), 5.74 (m, 2H, C**H**=CHCH$_2$NH, and N**H**CH), 6.08 (bs, 1H, N**H**CH$_2$).

^{13}C NMR ($CDCl_3$) δ 28.3, 32.6, 39.5, 49.8, 79.6, 124.6, 129.0, 142.5, 155.1, 174.7.

HRMS (ES) 249.1220 (M^++Na). $C_{11}H_{18}N_2O_3Na$ requires 249.1215.

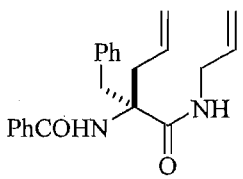
Data for (+/-)-**7.26**:

^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H, C(C**H**$_3$)$_3$), 2.19 (m, 1H, C**H**$_a$CH=), 2.82 (m, 1H, C**H**$_b$CH=), 4.27 (m, 1H, NHC**H**CH$_2$), 5.16 (t *J*=7.1Hz, 1H, CH=C**H**NH), 5.49 (bs, 1H, N**H**CHCH$_2$), 6.06 (m, 1H, C**H**=CHNH), 7.67 (bs, 1H, CH=CHN**H**).

^{13}C NMR δ 27.2, 28.3, 50.0, 79.8, 105.5, 124.7, 155.6, 170.1.

HRMS (ES) 235.1051 (M^++Na). $C_{10}H_{16}N_2O_3Na$ requires 235.1059.

Preparation of (1*R*)-*N*-(1-Allylcarbamoyl-1-benzyl-but-3-enyl)-benzamide 7.27.



Allylamine (11.5μL, 0.15mmol, 3 equiv) was dissolved in dry THF (2mL) and cooled to -78°C under argon. *n*-Butyl lithium (95μL, 0.15mmol, 3 equiv) was added and the mixture stirred at -78°C for 5 min. A solution of oxazolidinone (-)-**2.5** (20mg, 0.05mmol, 1 equiv) dissolved in dry THF (1mL) was then added and the solution allowed to warm to room temperature overnight. The reaction mixture was quenched with saturated aqueous NH_4Cl and successively washed with saturated aqueous $NaHCO_3$ (5mL), NaCl (5mL) and dried over $MgSO_4$. Purification by radial chromatography eluting with 1:3 ethyl acetate/petroleum ether yielded **7.27** (16mg, 91%) as a white solid. An analytical sample was obtained by the diffusion of petroleum ether into a solution of **7.27** dissolved in ethyl acetate. Crystals suitable for X-ray crystallography were obtained (see Appendix for data).

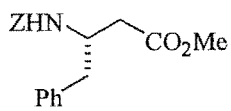
^{1}H NMR ($CDCl_3$) δ 2.77 (m, 1H, CC**H**$_a$CH=CH$_2$), 3.25 (m, CC**H**$_b$CH=CH$_2$), 3.37 (d *J*=13.9Hz, 1H, PhC**H**$_a$), 3.67 (d *J*=13.9Hz, 1H, PhC**H**$_b$), 3.88-4.01 (m, 2H, NHC**H**$_2$), 5.12-5.26 (m, 4H, 2 x CH=C**H**$_2$), 5.74 (m, 1H, CCH$_2$C**H**=), 5.84 (m, 1H, NHCH$_2$C**H**=), 6.51 (t *J*=4.5Hz, 1H, N**H**), 7.13 (m, 3H, Ph**H** and PhCON**H**), (NH couples long range to PhCH2)

7.22 (m, 3H, Ph**H**), 7.40 (t *J*=7.8Hz, 2H, Ph**H**), 7.49 (m, 1H, Ph**H**), 7.67 (dd *J*=1.2 and 8.5Hz, 2H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 40.0, 40.9, 42.5, 64.4, 117.1, 119.8, 126.8, 127.1, 128.3, 128.6, 130.1, 131.6, 132.3, 133.6, 135.0, 135.7, 167.3, 171.9.

HMRS (EI) 348.1829 (M^+). $C_{22}H_{24}N_2O_2$ requires 348.1838.

Preparation of (3*S*)-3-Benzyloxycarbonylamino-4-phenyl-butyric acid methyl ester (-)-7.34.



Et_3N (2.326mL, 16.72mmol, 1 equiv) and $ClCO_2Et$ (1.574mL, 16.72mmol, 1 equiv) were reacted with (*S*)-*N*-Cbz-phenylalanine (5g, 16.72mmol, 1 equiv) according to General Procedure Ja. The resulting diazoketone was dissolved in dry MeOH (80mL) and reacted with silver benzoate (421mg, 1.84mmol, 0.11 equiv) dissolved in Et_3N (6.746mL, 48.49mmol, 2.9 equiv), according to General Procedure Jb. Purification by column chromatography (EA/PE 1:3) gave (-)-**7.34** (5.294g, 97%) as a white solid

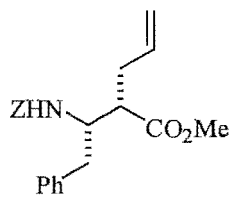
mp 50-52°, (lit. .[4] 53-55°).

^{1}H NMR ($CDCl_3$) δ 2.48 (dd *J*=16.1 and 5.8Hz, 1H, C**H**$_a$), 2.54 (dd *J*=16.1 and 5.4Hz, 1H, C**H**$_b$), 2.84 (dd *J*=13.8 and 7.8Hz, 1H, C**H**$_a$), 2.96 (dd *J*=13.4 and 6.2Hz, 1H, C**H**$_b$), 3.67 (s, 3H, O**Me**), 4.23 (m, 1H, NHC**H**), 5.07 (s, 2H, PhC**H**$_2$O), 5.29 (d *J*=8.8Hz, 1H, N**H**), 7.16-7.37 (m, 5H, Ph**H**).

FTIR (KBr) 3313, 2949, 1687cm^{-1}.

LRMS (ES) 328.1 (M^++H). $C_{19}H_{21}NO_4$ requires 328.1.

Preparation of (2*S*,1*S*)-2-(1-Benzyloxycarbonylamino-2-phenyl-ethyl)-pent-4-enoic acid methyl ester 7.35.



Anhydrous LiCl (565mg, 13.76mmol, 3 equiv), LDA (5.05mL of a 2M solution in THF, 10.09mmol, 2.2 equiv), and allyl bromide (1.589mL, 18.36mmol, 4 equiv) were reacted with a -78° C solution of (-)-**7.34** (1.5g, 4.59mmol) dissolved in THF (20mL) under nitrogen, according to General Procedure K. Purification by radial chromatography (EA/PE 1:4) gave **7.35** (1.068g, 64%) as a colourless oil that solidified on standing.

mp 46-48° C.

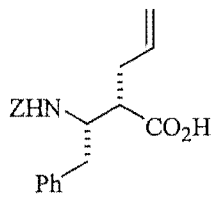
^{1}H NMR ($CDCl_3$) δ 2.29 (m, 1H, C**H**$_a$CH=CH_2), 2.41 (m, 1H, C**H**$_b$CH=CH_2), 2.61 (m, 1H, C**H**CO_2Me), 2.67 (dd *J*=13.7 and 8.3Hz, 1H, CHC**H**$_a$Ph), 2.91 (dd *J*=13.5 and 6.5Hz, 1H, CHC**H**$_b$Ph), 3.71 (s, 3H, O**Me**), 4.09 (m, 1H, NHC**H**), 4.99-5.14 (m, 2H, CH=C**H**$_2$), 5.08 (dd *J*=21.0 and 12.2Hz, 2H, PhC**H**$_2$), 5.65 (m, 1H, C**H**=CH_2), 5.81 (d *J*=9.3Hz, 1H, N**H**), 7.18-7.38 (m, 10H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 34.3, 40.6, 46.4, 51.6, 53.1, 66.5, 117.4, 126.5, 127.9, 127.9, 128.4, 129.2, 134.3, 136.6, 137.5, 155.9, 174.7.

FTIR (KBr) 3333, 2957, 1728, 1699, 1539cm^{-1}.

HRMS (ES) 368.1874 (M^++H) $C_{22}H_{25}NO_4$ requires 368.1862.

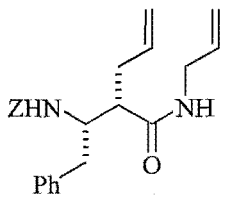
Preparation of (2*S*,1*S*)-2-(1-Benzyloxycarbonylamino-2-phenyl-ethyl)-pent-4-enoic acid 7.36.



1M aq. NaOH (1.74mL, 1.74mmol, 2 equiv) was reacted with methyl ester **7.35** (320mg, 0.87mmol, 1 equiv) dissolved in MeOH (15mL) according to General Procedure G, to give **7.36** (305mg, 99%) as a yellow oil.

^{1}H NMR ($CDCl_3$) δ 2.34 (m, 1H, C**H**$_a$CH=CH_2), 2.46 (m, 1H, C**H**$_b$CH=CH_2), 2.64 (m, 1H, C**H**CO_2H), 2.77 (dd *J*=13.7 and 8.8Hz, 1H, C**H**$_a$Ph), 2.95 (dd *J*=13.9 and 6.6Hz, 1H, C**H**$_b$Ph), 4.24 (m, 1H, NHC**H**), 5.00-5.15 (m, 4H, PhC**H**$_2$ and CH=C**H**$_2$), 5.68 (m, 1H, C**H**=CH_2), 5.71 (d *J*=9.8Hz, 1H, N**H**), 7.14-7.37 (m, 10H, Ph**H**).

Preparation of (2*S*,1*S*)-(2-Allylcarbamoyl-1-benzyl-pent-4-enyl)-carbamic acid benzyl ester 7.37.



EDCI (232mg, 1.12mmol, 1.3 equiv), HOBt (190mg, 1.3mmol, 1.5 equiv), allylamine (0.073mL, 1.3mmol, 1.5 equiv) and diisopropylethylamine (0.205mL, 0.95mmol, 1.1 equiv) were added to a solution of **7.36** (305mg, 0.86mmol, 1 equiv) dissolved in CH_2Cl_2 (10mL) under argon. The mixture was stirred at rt under argon for 16h. Purification by radial chromatography (EA/PE 1:4) gave **7.37** (261mg, 74%) as a white solid.

mp 168-169°C.

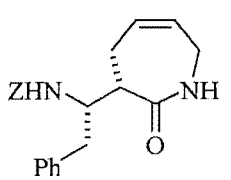
^{1}H NMR ($CDCL_3$) δ 2.13 (m, 1H, C**H**$CH_2CH{=}CH_2$), 2.27 (m, 1H, CHC**H$_a$**$CH{=}CH_2$), 2.42 (m, 2H, CHC**H$_b$**$CH{=}CH_2$), 2.62 (dd *J*=13.7 and 9.2Hz, 1H, CHC**H$_a$**Ph), 3.05 (dd *J*=13.9 and 6.1Hz, 1H, CHC**H$_b$**Ph), 3.89 (m, 2H, NHC**H$_2$**$CH{=}CH_2$), 4.06 (m, 1H, NHC**H**), 4.99 (m, 2H, NHC**H$_2$**$CH{=}CH_2$), 5.09 (dd *J*=15.6 and 12.7Hz, 2H, $CHCH_2CH{=}C$**H$_2$**), 5.20 (m, 2H, PhC**H$_2$**O), 5.47 (br s, 1H, N**H**CH_2), 5.64 (m, 1H, $CHCH_2C$**H**$=CH_2$), 5.84 (m, 1H, $NHCH_2C$**H**$=CH_2$), 6.50 (d *J*=8.8Hz, 1H, N**H**CH), 7.11-7.36 (m, 10H, Ph**H**).

^{13}C NMR δ 34.9, 40.4, 41.8, 47.2, 53.8, 66.3, 117.1, 117.7, 126.6, 127.8, 127.9, 128.4, 128.6, 129.0, 133.7, 134.7, 136.8, 138.1, 156.2, 173.6.

FTIR (KBr) 3433, 1693, 1643, 1551, 1265cm^{-1}.

HRMS (ES) 393.2176 (M^++H). $C_{24}H_{29}N_2O_3$ requires 393.2178.

Preparation of (3*S*,1*S*)-[1-(2-Oxo-2,3,4,7-tetrahydro-1H-azepin-3-yl)-2-phenyl-ethyl]-carbamic acid benzyl ester 7.38.



Catalyst **1.42** (9mg,, 5mol%), dissolved in *dry degassed* benzene (0.5mL), was added to a solution of diene **7.37** (70mg, 0.18mmol, 1 equiv) dissolved in *dry degassed* benzene

(3mL) under argon, according to General Procedure E. The mixture was then stirred at 50° C for 2h. Purification by radial chromatography (EA/PE 1:1) gave **7.38** (58mg, 89%) as a white solid.

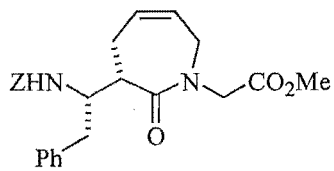
^{1}H NMR ($CDCl_3$) d 2.15 (bd *J*=9.0Hz, 1H, CHC**H**$_a$CH=), 2.47 (m, 1H, CHC**H**$_b$CH=), 2.95 (m, 2H, C**H**CH$_2$CH= and PhC**H**$_a$), 3.06 (dd *J*=6.3 and 13.2Hz, 1H, PhC**H**$_b$), 3.28 (m, 1H, NHC**H**$_a$CH=), 3.81 (bd *J*=7.6Hz, 1H, NHC**H**$_b$CH=), 3.99 (m, 1H, NHC**H**), 5.08 (dd *J*=12.2 and 24.4Hz, 2H, PhC**H**$_2$O), 5.59 (m, 1H, NHCH$_2$C**H**=), 5.68 (m, 1H, CHCH$_2$C**H**=), 6.16 (bs, 1H, N**H**CH$_2$), 6.58 (d *J*=9.8Hz, 1H, N**H**CH), 7.20-7.37 (m, 10H, Ph**H**).

^{13}C NMR ($CDCl_3$) d 29.5, 39.2, 39.9, 40.6, 55.5, 66.4, 124.3, 126.4, 127.8, 127.9, 128.4, 128.6, 129.1, 130.5, 136.7, 138.6, 156.5, 177.5.

FTIR (KBr) 3275, 2924, 1699, 1651cm^{-1}.

HRMS (ES) 365.1862 (M^++H). $C_{22}H_{25}N_2O_3$ requires 365.1865.

Preparation of (3*S*,1*S*)-[3-(1-Benzyloxycarbonylamino-2-phenyl-ethyl)-2-oxo-2,3,4,7-tetrahydro-azepin-1-yl]-acetic acid methyl ester 7.39.



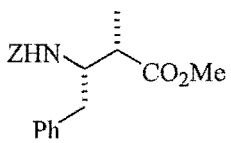
NaH (13mg of 60% in oil, 0.33mmol, 2 equiv) was slowly added to a solution of **7.38** (60mg, 0.165mmol) dissolved in dry CH_3CN (5mL), and the mixture was stirred at rt for 16h. After filtering, the solvent was removed under reduced pressure and the residue purified by radial chromatography (EA/PE 1:1) to yield **7.39** (66mg, 92%) as a white solid.

^{1}H NMR ($CDCl_3$) δ 2.15 (m, 1H, CHC**H**$_a$CH=), 2.47 (m, 1H, CHC**H**$_b$CH=), 2.92 (dd *J*=10.3 and 13.2Hz, 1H, PhC**H**$_a$), 3.03 (dd *J*=5.8 and 13.2Hz, 1H, PhC**H**$_b$), 3.07 (dt *J*=2.9 and 13.2Hz, 1H, NC**H**$_a$CH=), 3.22 (dd *J*=7.8 and 17.6Hz, 1H, CHC**H**CO), 3.78 (s, 3H, O**Me**), 3.85 (d *J*=17.6Hz, 1H, C**H**$_a$CO$_2$Me), 3.96 (m, 1H, NHC**H**), 4.21 (dt *J*=2.9 and 17.6Hz, 1H, NHC**H**$_b$CH=), 4.56 (d *J*=17.1Hz, 1H, C**H**$_b$CO$_2$Me), 5.09 (dd *J*=12.5 and 20.8Hz, 2H, PhC**H**$_2$O), 5.61 (m, 1H, NHCH$_2$C**H**=), 5.71 (m, 1H, CHCH$_2$C**H**=), 6.59 (d *J*=10.3Hz, 1H, N**H**CH), 7.20-7.37 (m, 10H, Ph**H**).

^{13}C NMR δ 30.0, 39.9, 40.6, 47.3, 49.6, 52.2, 55.9, 66.3, 123.3, 126.4, 127.7, 127.8, 128.4, 128.6, 129.2, 131.2, 136.7, 138.6, 156.5, 169.6, 175.4.

HRMS (ES) 437.2083 (M^++H). $C_{25}H_{29}N_2O_5$ requires 437.2076.

Preparation of (3*S*,2*S*)-3-Benzyloxycarbonylamino-2-methyl-4-phenyl-butyric acid methyl ester 7.40.

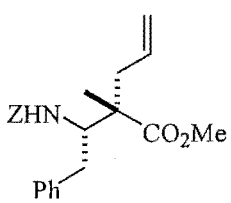


Anhydrous LiCl (753mg, 18.35mmol, 3 equiv), LDA (6.73mL of a 2M solution in THF, 13.5mmol, 2.2 equiv), and MeI (1.524mL, 24.5mmol, 4 equiv) were reacted with a -78° C solution of (-)-**7.34** (2g, 6.12mmol) dissolved in THF (30mL) under nitrogen, according to General Procedure K. Purification by radial chromatography (EA/PE 1:4) gave **7.40** (1.998g, 96%) as a colourless oil.[4]

^{1}H NMR ($CDCl_3$) δ 1.21 (d *J*=7.3Hz, 3H, C**H**$_3$), 2.65 (m, 1H, C**H**CH$_3$), 2.72 (dd *J*=13.7 and 8.1Hz, 1H, PhC**H**$_a$), 2.90 (dd *J*=13.7 and 6.9Hz, 1H, PhC**H**$_b$), 3.71 (s, 1H, OC**H**$_3$), 4.02 (m, 1H, NHC**H**), 5.07 (dd *J*=16.3 and 12.5Hz, 2H, OC**H**$_2$), 5.68 (d *J*=10.3Hz, 1H, N**H**), 7.17-7.39 (m, 10H, Ph**H**).

LRMS (ES) 342.3 (M^++H). $C_{20}H_{24}NO_4$ requires 342.3.

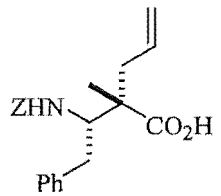
Preparation of (2*S*,1*S*)-2-(1-Benzyloxycarbonylamino-2-phenyl-ethyl)-2-methyl-pent-4-enoic acid methyl ester 7.41.



Anhydrous LiCl (685mg, 16.7mmol, 3 equiv), LDA (6.13mL of a 2M solution in THF, 12.25mmol, 2.2 equiv), and allyl bromide (1.928mL, 22.28mmol, 4 equiv) were reacted with a -78° C solution of **7.40** (1.9g, 5.57mmol) dissolved in THF (25mL) under nitrogen, according to General Procedure K. Purification by radial chromatography (EA/PE 1:4) gave **7.41** (819mg, 39%) as a colourless oil.[4]

^{1}H NMR ($CDCl_3$) δ 1.29 (s, 3H, CC**H$_3$**), 2.29 (dd *J*=13.9 and 7.5Hz, 1H, C**H$_a$**CH=CH$_2$), 2.41 (dd *J*=13.6 and 10.7Hz, 1H, PhC**H$_a$**CH), 2.55 (dd *J*=13.7 and 7.4Hz, 1H, C**H$_b$**CH=CH$_2$), 3.03 (dd *J*=13.7 and 3.4Hz, 1H, PhC**H$_b$**CH), 3.66 (s, 3H, O**Me**), 4.01 (td *J*=10.7 and 3.4Hz, 1H, NHC**H**), 4.92 (dd$_{AB}$ *J*=53.5 and 12.5Hz, 2H, PhC**H$_2$**O), 5.06 (m, 2H, CH=C**H$_2$**), 5.38 (d *J*=10.7Hz, 1H, N**H**), 5.71 (m, 1H, C**H**=CH$_2$), 7.17-7.39 (m, 10H, Ph**H**).

Preparation of (2*S*,1*S*)-2-(1-Benzyloxycarbonylamino-2-phenyl-ethyl)-2-methyl-pent-4-enoic acid 7.42.

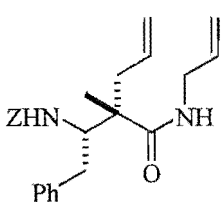


1M aq. NaOH (2.36mL, 2.36mmol, 2 equiv) was reacted with methyl ester **7.41** (450mg, 1.18mmol) dissolved in MeOH (20mL) according to General Procedure G, to give **7.42** (420mg, 97%) as a yellow oil.

1H NMR ($CDCl_3$) d 1.44 (s, 3H, CCH_3), 2.36 (dd *J*=13.7 and 7.8Hz, 1H, $CH_aCH=CH_2$, 2.51 (dd *J*=13.9 and 11.5Hz, 1H, CHC**H$_a$**Ph), 2.62 (dd *J*=13.7 and 7.3Hz, 1H, C**H$_b$**CH=CH$_2$), 3.07 (dd *J*=13.9 and 3.4Hz, 1H, CHC**H$_b$**Ph), 4.11 (td *J*=11.1 and 3.4Hz, 1H, NHC**H**), 4.87 (dd *J*=74.9 and 12.2Hz, 2H, PhC**H$_2$**O), 5.12 (m, 2H, CH=C**H$_2$**), 5.78 (m, 1H, C**H**=CH$_2$), 7.17-7.39 (m, 10H, Ph**H**).

HRMS (ES) 368.1864 (M^++H). $C_{22}H_{26}NO_4$ requires 368.1862.

Preparation of (2*S*,1*S*)-(2-Allylcarbamoyl-1-benzyl-2-methyl-pent-4-enyl)-carbamic acid benzyl ester 7.43.



EDCI (118mg, 0.57mmol, 1.3 equiv), HOBt (97mg, 0.66mmol, 1.5 equiv), allylamine (0.037mL, 0.66mmol, 1.5 equiv) and diisopropylethylamine (0.104mL, 0.48mmol, 1.1

equiv) were added to a solution of **7.42** (160mg, 0.44mmol) dissolved in CH_2Cl_2 (5mL) under argon. The mixture was stirred at rt under argon for 16h. Purification by radial chromatography (EA/PE 1:3) gave **7.43** (128mg, 71%) as a white solid. An analytical sample was obtained by the diffusion of petroleum ether into a solution of (+/-)-**3.12** dissolved in ethyl acetate. Crystals suitable for X-ray crystallography were obtained (see Appendix for data).

mp 99-101°C

^{1}H NMR ($CDCl_3$) δ 1.28 (s, 3H, CC**H**$_3$), 2.27 (dd *J*=13.6 and 7.8Hz, 1H, C**H**$_a$CH=CH_2), 2.48 (dd *J*=13.6 and 10.7Hz, 1H, PhC**H**$_a$CH), 2.58 (dd *J*=13.5 and 6.5Hz, 1H, C**H**$_b$CH=CH_2), 2.98 (dd *J*=13.7 and 3.4Hz, 1H, PhC**H**$_b$CH), 3.88 (m, 3H, NHC**H** and NHC**H**$_2$), 4.86 (d *J*=12.7, 2H, PhC**H**$_a$O), 4.94 (d *J*=12.7Hz, 1H, PhC**H**$_b$O), 5.06-5.22 (m, 4H, 2xCH=C**H**$_2$), 5.71 (m, 1H, CCH_2C**H**=CH_2), 5.84 (m, 2H, N**H** CH_2 and $NHCH_2$C**H**=CH_2), 6.09 (d *J*=10.3Hz, 1H, N**H**), 7.16-7.37 (m, 10H, Ph**H**).

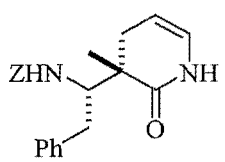
^{13}C NMR ($CDCl_3$) δ 19.9, 37.8, 41.9, 42.0, 48.8, 59.0, 66.1, 116.8, 119.1, 126.3, 127.6, 127.7, 128.2, 128.3, 129.3, 133.3, 136.9, 138.2, 156.2, 175.4.

FTIR (KBr) 3279, 1709, 1634, 1547cm^{-1}.

HRMS (ES) 407.2334 (M^++1). $C_{25}H_{31}N_2O_3$ requires Calcd 407.2335.

Micro. Calcd for $C_{25}H_{30}N_2O_3$, C, 73.86; H, 7.44; N, 6.89. Found: C, 73.56; H, 7.54; N, 6.98.

Preparation of (1*S*,3*S*)-[1-(3-Methyl-2-oxo-1,2,3,4,-tetrahydro-pyridin-3-yl)-2-phenyl-ethyl]-carbamic acid benzyl ester 7.45.



Catalyst **1.42** (8mg,, 5mol%), dissolved in *dry degassed* benzene (0.5mL), was added to a solution of diene **7.43** (75mg, 0.185mmol)) dissolved in *dry degassed* benzene (3mL) under argon, according to General Procedure E. The mixture was then refluxed at 80° C for 4h. Purification by radial chromatography (EA/PE 1:3) **7.45** (59mg, 88%) as a white solid.

^{1}H NMR ($CDCl_3$) δ 1.31 (s, 3H, CC**H$_3$**), 2.03 (dd *J*=17.1 and 5.6Hz, 1H, C**H$_a$**CH=CH), 2.62 (bd *J*=17.1Hz, 1H, C**H$_b$**CH=CH), 3.02 (m, 1H, PhC**H$_a$**CH), 3.14 (m, 1H, PhC**H$_b$**CH), 4.10 (m, 1H, NHC**H**), 4.80 (d *J*=12.7, 1H, PhC**H$_a$**O), 4.94 (d *J*=12.7Hz, 1H, PhC**H$_b$**O), 5.07 (m, 2H, CCH_2C**H**=CH and N**H**), 5.98 (m, 1H, NHC**H**=), 6.99 (m, 1H, N**H**), 7.11-7.32 (m, 10H, Ph**H**).

^{13}C NMR ($CDCl_3$) δ 20.3 (C**C**H_3), 31.1 (**C**H_2CH=CH), 37.7 (**C**H_2Ph), 45.1 (**C**CH_3), 57.5 (NH**C**H), 66.3 (Ph**C**H_2O), 103.9 (**C**H=CHNH), 123.7 (=**C**HNH), 126.2, 127.7, 127.8, 128.2, 128.4, 129.2, 136.6, 138.9, 156.6 (O**C**ONH), 174.7 (**C**ONH).

HRMS (ES) 365.1866 (M^++1). $C_{22}H_{25}N_2O_3$ requires 365.1865.

8.8 References for Experimental

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APPENDIX

X-RAY CRYSTALLOGRAPHIC DATA

Crystallography

Tables 1 – 3 list crystal data for the eleven crystal structures discussed in this thesis. Selected bond angles and torsion angles are listed in the discussion of the structures, and the remaining distances and angles, as well as atom coordinates, anisotropic displacement parameters and hydrogen atom coordinates are available from the Chemistry Department of the University of Canterbury.

All measurements were made with a Seimens CCD area detector using graphite monochromised Mo Kα (λ = 0.71073 Å) radiation at the temperature indicated in the following tables. The data reduction was performed using SAINT.[1] Intensities were corrected for Lorentz and polarization effects and for absorption using SADABS. Space groups were determined from systematic absences and checked for higher symmetry. The structures were solved by direct methods using SHELXS, and refined on F^2 using all data using full-matrix least squares procedures with SHELXL-97. All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were fixed in idealized positions. Absolute structure determinations were based on the Flack parameter. In all cases, final Fourier syntheses showed no significant residual electron density in chemical sensible positions.

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Table 1. Crystal data and X-ray experimental details for (-)-**2.5**, (+)-**2.9**, (+)-**2.12**, **2.16**, **3.12**.

Compound	(-)-2.5	(+)-2.9	(+)-2.12	2.16	3.12
Data Collection Device	CCD	CCD	CCD	CCD	CCD
Emperical Formula	$C_{27}H_{23}NO_3$	$C_{21}H_{21}NO_3$	$C_{21}H_{23}NO_3$	$C_{20}H_{18}BrNO_3$	$C_{26}H_{25}NO_3S$
Formula Weight	409.46	335.39	337.40	400.26	431.53
Temperature (K)	158(2)	293(2)	168(2)	168(2)	168(2)
Crystal System	Orthorhombic	Orthorhombic	Orthorhombic	Orthorhombic	Monoclinic
Space Group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_1/c$
Unit Cell Dimensions: a (Å)	7.091(14)	11.000(4)	7.582(7)	6.271(11)	8.762(2)
b (Å)	16.52(3)	13.360(4)	13.621(16)	15.83(3)	19.013(5)
c (Å)	17.85(3)	24.060(4)	17.52(2)	17.57(3)	13.712(4)
α (°)	90	90	90	90	90
β (°)	90	90	90	90	99.836(4)
γ (°)	90	90	90	90	90
Volume ($Å^3$)	2091(7)	3535.9(18)	1809(3)	1745(5)	2250.7(11)
Z	4	8	4	4	4
Density (calculated) (Mg/m^3)	1.301	1.260	1.239	1.523	1.273
Absorption coefficient (mm^{-1})	0.085	0.084	0.082	2.374	0.171
F(000)	864	1424	720	816	912
Crystal Size (mm^3)		0.75x0.65x0.3	0.75x0.12x0.10		0.75x0.55x0.25
Theta range for data collection (°)	1.68 to 26.41	2.94 to 26.40	1.89 to 26.40	2.32 to 26.79	1.85 to 26.40
Reflections collected	7583	13962	7931	6974	29292
Independent reflections [R(int)]	3639	6730	3582	3395	4577
Data / restraints / parameters	3639 / 0 / 271	6730 / 0 / 453	3582 / 0 / 227	3395 / 0 / 226	4577 / 0 / 289
Goodness-to-fit on F^2	1.071	1.054	0.790	1.025	1.033
R_1 [$I>2\sigma(I)$]	0.0712	0.0398	0.0432	0.0315	0.0396
wR_2 (all data)	0.1648	0.0779	0.0867	0.0745	0.1122

Table 2. .Crystal data and X-ray experimental details for (-)-**3.24**, (+)-**3.13**, (-)-**3.17**, (+)-**5.40a**, **7.27**.

Compound	(-)-**3.24**	(+)-**3.13**	(-)-**3.17**	(+)-**5.40a**	**7.27**
Structure					
Data Collection Device	CCD	CCD	CCD	CCD	CCD
Emperical Formula	$C_{17}H_{23}NO_3S$	$C_{25}H_{21}NO_3$	$C_{20}H_{19}NO_3$	$C_{21}H_{27}NO_5$	$C_{22}H_{24}N_2O_4$
Formula Weight	321.42	383.43	321.36	373.44	348.43
Temperature (K)	168(2)	293(2)	168(2)	168(2)	566(2)
Crystal System	Monoclinic	Orthorhombic	Monoclinic??	Orthorhombic	Orthorhombic
Space Group	$P2_1$	$P2_12_12_1$		$P2_12_12_1$	$P2_12_12_1$
Unit Cell Dimensions: a (Å)	9.847(4)	8.612(4)	19.516(4)	5.773(6)	9.806(4)
b (Å)	9.356(4)	9.467(6)	7.2111(15)	10.373(13)	10.895(5)
c (Å)	10.168(4)	24.782(15)	24.682(5)	33.36(4)	18.899(9)
α (°)	90	90	90	90	90
β (°)	112.216(5)	90	103.468	90	90
γ (°)	90	90	90	90	90
Volume ($Å^3$)	867.2(6)	2020(2)	3378.1(12)	1998(4)	2019.2(16)
Z	2	4	8	4	2
Density (calculated) (Mg/m^3)	1.231	1.261	1.264	1.242	1.146
Absorption coefficient (mm^{-1})	0.198	0.083	0.085	0.088	0.074
F(000)	344	800	1360	800	744
Crystal Size (mm^3)	0.12x0.44x0.5	0.54x0.43x0.25	0.77x0.55x0.48	0.55x0.38x0.33	
Theta range for data collection (°)	2.16 to 26.41	2.30 to 25.40	2.15 to 26.43	2.31 to 26.41	2.16 to 26.36
Reflections collected	11239	8763	20377	8924	4899
Independent reflections [R(int)]	3504	4006	3414	3563	3645
Data / restraints / parameters	3504 / 0 / 199	4006 / 0 / 257	3414 / 0 / 219	3563 / 0 / 244	3645 / 0 / 235
Goodness-to-fit on F^2	0.918	1.022	1,097	0.905	0.983
R_1 [$I>2\sigma(I)$]	0.0279	0.0442	0.0436	0.0322	0.0681
wR_2 (all data)	0.0559	0.1104	0.1071	0.0685	0.1621

Table 3. .Crystal data and X-ray experimental details for **7.43**.

Compound	**7.43**
Structure	
Data Collection Device	CCD
Emperical Formula	$C_{25}H_{30}N_2O_3$
Formula Weight	406.51
Temperature (K)	163(2)
Crystal System	Orthorhombic
Space Group	$P2_12_12_1$
Unit Cell Dimensions: a (Å)	13.460(2)
b (Å)	18.666(3)
c (Å)	27.632(4)
α (°)	90
β (°)	90
γ (°)	90
Volume ($Å^3$)	6942(2)
Z	12
Density (calculated) (Mg/m^3)	1.167
Absorption coefficient (mm^{-1})	0.077
F(000)	2616
Crystal Size (mm^3)	0.50x0.20x0.08
Theta range for data collection (°)	1.68 to 26.47
Reflections collected	53219
Independent reflections [R(int)]	14198
Data / restraints / parameters	14198 / 546 / 838
Goodness-to-fit on F^2	0.941
R_1 [$I>2\sigma(I)$]	0.0755
wR_2 (all data)	0.2411